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ACTA PHYSIOLOGICA SCANDINAVICA

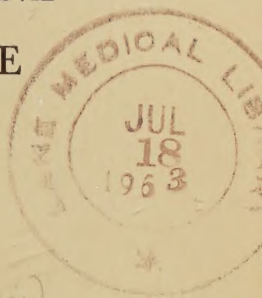
VOL. 58. SUPPLEMENTUM 204

CATECHOLAMINES
AND 5-HYDROXYTRYPTAMINE
IN MORPHINE TOLERANCE
AND WITHDRAWAL

BY

LARS-M. GUNNE

(analyt.)



STOCKHOLM 1963

ACTA PHYSIOLOGICA SCANDINAVICA

Vol. 58. Supplementum 204

FROM THE DEPARTMENT OF PHYSIOLOGY AND THE
DEPARTMENT OF ALCOHOL RESEARCH

KAROLINSKA INSTITUTET,
STOCKHOLM 60, SWEDEN

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Printed in Sweden

TRYCKERI AKTIEBOLAGET THULE
STOCKHOLM 1963

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AKADEMISK AVHANDLING

SOM MED TILLSTÅND AV

KUNGL. KAROLINSKA INSTITUTETS LÄRARKOLLEGIUM

FÖR ERNÅENDE AV MEDICINE DOKTORSGRAD

OFFENTLIGEN FÖRSVARAS I

FYSIOLOGISKA INSTITUTIONENS FÖRELÄSNINGSSAL, KAROLINSKA INSTITUTET,

TISDAGEN DEN 26 MARS 1963 KL. 9 F. M.

AV

LARS-M. GUNNE

MED. LIC.

STOCKHOLM 1963



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INTRODUCTION

It is an old clinical observation that morphine addicts can withstand large amounts of the drug without showing conspicuous manifestations of physical or mental alteration. Withdrawal of morphine, however, precipitates the characteristic abstinence syndrome, which shows many features indicative of a gross disturbance of the autonomic nervous system, together with excitation which is sometimes exaggerated to the point of delirium. After a few stormy days, the recovery process becomes evident and the characteristic syndrome generally subsides in about ten days.

KOLB and HIMMELSBACH (1938) have developed a quantitative method for the scoring of all measurable abstinence manifestations in man. Some of these signs and symptoms are: restlessness, rise in respiratory rate and pulse rate, rise in blood pressure, fever and rise in basal metabolism, rise in blood sugar, dilated pupils, tremor, pilo-erection, reduced weight and calory intake, insomnia, yawning, rhinorrhea, lacrimation, perspiration, emesis and diarrhea.

An important contribution to the study of abstinence phenomena was offered by the observation of ISBELL and FRASER (1950) that a complete, but transient, abstinence syndrome was produced in morphine-dependent addicts by injection of N-allylnormorphine (nalorphine), thus unmasking the patients' dependence on the drug.

Corresponding symptoms of excitation with several autonomic manifestations have been described in dogs and in some rat strains after withdrawal from long-term administration of morphine (JOEL and ETTINGER 1926, PLANT and PIERCE 1928).

The vegetative excitation syndrome is a well recognized phenomenon in brain stimulation and ablation studies on various experimental animals. Such experiments have indicated the importance of limbic and hypothalamic brain areas in modulating autonomic and somatic activity during behavioral reactions (for references see KAADA 1951, HESS 1954).

The discovery of a series of biologically active substances in those areas of the brain stem¹ where autonomic functions had been localized, stimulated the rapid evolution of neuropharmacology during the last decade. Soon after the localization of high concentrations of noradrenaline (VOGT 1954) and 5-hydroxytryptamine (serotonin) (AMIN, CRAWFORD and GADDUM

¹ In this paper the brain stem is defined as medulla oblongata, pons, midbrain and diencephalon.

1954) to certain areas of the brain stem, the discovery was made that the brain content of these monoamines could be influenced by various drugs. The dramatic changes produced by tranquilizers of the reserpine group and the centrally stimulating monoamine oxidase inhibitors have been extensively studied during the last few years, notably with regard to their implications for the function of brain monoamines (for references see BRODIE, SPECTOR and SHORE 1959 a).

The present investigation was prompted by the findings of VOGT (1954) that a single injection of morphine elicits excitation and an increased sympathetic activity in cats, together with a depletion of noradrenaline from the hypothalamic and midbrain stores. These acute effects of morphine in cats actualized the related problem of morphine abstinence and the possible neurochemical mechanisms underlying this syndrome. A series of reports dealing with this problem has appeared earlier from this laboratory (GUNNE 1959, 1960, 1961, 1962 a, b); the present paper is an extension of these earlier studies. It was carried out according to the following general plan:

At first a study was made of the effects of acute and chronic morphine administration, as well as of withdrawal and nalorphine-induced abstinence on the general behavior of some laboratory animals. Special attention was paid to species differences observed between dogs, rats and cats (Chapter III). A study was then made of the effects of chronic morphine treatment and abstinence on the brain content of noradrenaline, dopamine and 5-hydroxytryptamine (Chapter IV) and on the adrenal gland content of adrenaline and noradrenaline (Chapter V). In order to obtain additional information concerning the rate of synthesis and liberation of the monoamines, a similar study was finally made of the urinary output of adrenaline, noradrenaline and 5-hydroxyindoleacetic acid (5-HIAA), the main urinary metabolite of 5-hydroxytryptamine (Chapter VI).

CHAPTER I

EARLIER LITERATURE

Occurrence of catecholamines in brain, adrenals and urine

Adrenaline, isolated in 1901 by TAKAMINE, is the main active principle in the adrenal medulla of mammals. It was synthesized in 1904 by STOLZ, as was also noradrenaline. Adrenaline was long thought to be the main sympathetic transmitter, as suggested by ELLIOTT (1905).

The physiological importance of noradrenaline was clarified much later (EULER 1946), when it was shown that the active sympathomimetic principle consists chiefly of noradrenaline. Up to that time all measurements of catecholamines discharged from sympathetic nerve terminals had been measured as "adrenaline", generally by bioassay methods.

In 1947 HOLTZ, CREDNER and KRONEBERG discovered the presence of noradrenaline in normal urine. The main source of the urinary noradrenaline, secreted under resting conditions, is probably the adrenergic nerves, while the small amounts of adrenaline originate from the adrenal glands (EULER 1956, p. 287). A massive discharge, mainly of adrenaline, may occur in emergency states.

EULER (1946) first demonstrated the presence of small amounts of catecholamines, mainly noradrenaline, in the brain. This observation was extended by VOGT (1954), who found that the brain catecholamines were unevenly distributed. The highest concentrations were found in the hypothalamus and somewhat less in the grey stratum around the aqueduct. The concentration of noradrenaline in the grey matter of the telencephalon was only about 5 per cent of that of the hypothalamus, while the medullated fibres contained still less. The very high concentrations of noradrenaline in certain parts of the brain suggested a possible transmitter function of noradrenaline in the brain tissue, in addition to its role as a transmitter at vasomotor endings. Recently the catecholamine stores of hypothalamic nuclei have been visualized by histochemical methods, through the work of CARLSSON, FALCK and HILLARP (1962).

The presence of dopamine (3-hydroxytyramine) in the mammalian brain was suggested by MONTAGU (1957) and proved, through independent work, by EULER (1958) and CARLSSON *et al.* (1958). BERTLER and

ROSENGREN (1959) investigated the distribution of dopamine in the brain and found high concentrations in the central ganglia of the telencephalon, notably the caudate nucleus. The substance was tentatively correlated with locomotor functions of the extrapyramidal system. It is generally agreed that the dopamine of the brain serves also as a precursor of noradrenaline.

The relative contribution of adrenaline to the mammalian brain "sympathin" has been a matter of differences in opinion. HOLTZ (1950) and VOGT (1954), using bioassay methods, obtained relative values for adrenaline between 8 and 25 per cent (of the sum of noradrenaline and adrenaline). MONTAGU (1956 a) found 34 per cent adrenaline in the rat brain, while BERTLER and ROSENGREN (1959) were unable to verify these high adrenaline levels in mammalian brains and found appreciable amounts only in the brains of amphibia.

Occurrence of 5-hydroxytryptamine in brain and 5-hydroxyindoleacetic acid in urine

The presence of 5-hydroxytryptamine (5-HT, serotonin, enteramine) in the brain was first reported by TWAROG and PAGE (1953). AMIN, CRAWFORD and GADDUM (1954) reported a distribution of this substance in the brain very similar to that of noradrenaline, the largest amounts being stored in the hypothalamus. TITUS and UDENFRIEND (1954) and ERSFAMER (1954 a) have investigated the fate of 5-hydroxytryptamine in the organism. They found that the most important metabolic route was the oxidative deamination by monoamine oxidase, with 5-hydroxyindoleacetic acid (5-HIAA) as the metabolic end-product, appearing in the urine. Most of the 5-hydroxytryptamine in the body is stored in the intestinal mucosa and the blood platelets (FELDBERG and TOH 1953, GADDUM 1954), from which the bulk of the urinary 5-HIAA is derived.

The brain stores of 5-hydroxytryptamine, although quantitatively insignificant, have nevertheless received much attention. It was speculated (GADDUM 1954, WOOLLEY and SHAW 1954), that this substance would have a role in brain function, possibly as a neurohumoral agent (WELSH 1954, BRODIE, PLETSCHER and SHORE 1955). Following the release of 5-hydroxytryptamine, it is attacked by the enzyme monoamine oxidase, which is present in the brain and 5-HIAA is formed. Since this acid passes slowly through the blood-brain barrier, the 5-HIAA of the brain is a measure of the local release of 5-hydroxytryptamine (ROOS and WERDINIUS 1962).

Brain monoamines and central excitation

In 1952 ZELLER and BARSKY, studying the monoamine oxidase inhibiting properties of some hydrazine derivatives, suggested that the effect of these drugs on the monoamines might have some relation to "the sympathetic and general mental stimulation observed". A few years later, when the monoamines of the brain had been mapped out, this suggestion gained new interest.

During the last few years several compounds have been developed which act upon the synthesis, storage, and break-down of the brain monoamines. By interference with any one of these mechanisms it has been possible to produce states of excitement in various laboratory animals. Corresponding signs have also been found in man (DEGKWITZ *et al.* 1960).

Synthesis: According to a now widely accepted theory, first suggested by BLASCHKO 1939, the biosynthesis of the catecholamines occurs according to the following scheme:

tyrosin $\rightarrow$ DOPA $\rightarrow$ dopamine $\rightarrow$ noradrenaline $\rightarrow$ adrenaline

The enzyme DOPA-decarboxylase is present in the brain (HOLTZ and WESTERMANN 1956) where it converts DOPA (3,4-dihydroxyphenylalanine) to dopamine. Probably the same enzyme (ROSENGREN 1960 a) attacks also 5-hydroxytryptophan (5HTP), the precursor of 5-hydroxytryptamine. The decarboxylation is a rapid process (UDENFRIEND 1959) which takes place as soon as the amino acids are present in the brain. The administration of 5-HTP or DOPA thus affords a means of increasing the rate of synthesis of 5-hydroxytryptamine or the catecholamines in the brain (UDENFRIEND, BOGDANSKI and WEISSBACH 1956, CARLSSON *et al.* 1958). The amines themselves do not readily pass the blood-brain barrier (WEIL-MALHERBE, AXELROD and TOMCHICK 1959, WILSON and BRODIE 1961).

When 5-HTP is administered to rats there is an increase in the 5-hydroxytryptamine content of the brain, and at the same time a state of excitation is noted (UDENFRIEND, WEISSBACH and BOGDANSKI 1957). Following administration of DOPA a corresponding increase of brain dopamine is seen together with central excitation (CARLSSON *et al.* 1958, BERTLER 1960).

Attempts to decrease the synthesis of brain monoamines by inhibition of the decarboxylating enzyme have not been successful *in vivo* (BRODIE *et al.* 1962). Some of the potent *in vitro* inhibitors have been shown to act by other mechanisms, as releasers of central catecholamines (UDENFRIEND, CONNAMACHER and HESS 1961).

Storage: The monoamines of the brain are stored in an inactive form. Following liberation they are attacked by the catabolizing enzymes. Four groups of agents which act upon the size of these stores are exemplified:

1) A release can be produced by intense stimulation through nervous mechanisms. This seems to be the mechanism of action of a series of different compounds, like insulin, nicotine and morphine (VOGT 1954). The common denominator of these compounds is the stimulation of sympathetic nerves.

The stimulation effects a reduction of the catecholamine level of the brain, while 5-hydroxytryptamine remains unaffected (PAASONEN and VOGT 1956). The catecholamine level is rapidly restored when the stimulation is ended.

2) Release can be effected by interfering with the amine-binding capacity of the tissues. An example of an agent which operates by this mechanism is reserpine. In contrast to the agents in group 1 (above) reserpine produces a long-lasting depletion of the brain content of both 5-hydroxytryptamine (PLETSCHER, SHORE and BRODIE 1956 b, PAASONEN and VOGT 1956), noradrenaline (HOLZBAUER and VOGT 1956) and dopamine (CARLSSON *et al.* 1958, WEIL-MALHERBE and BONE 1958). It is well known that this depletion is accompanied by marked sedation and loss of spontaneous activity.

The activity can be restored by DOPA but not by 5-hydroxytryptophan (CARLSSON, LINDQVIST and MAGNUSSON 1957), thus suggesting that reserpine may act by producing a lack of central transmitter. There are also, however, other theories regarding these mechanisms, which involve 5-hydroxytryptamine (BRODIE *et al.* 1957, GESSA *et al.* 1962) or its deaminated end-product (HOLTZ *et al.* 1957) as the centrally acting sedative substance.

3) A third way of releasing the brain stores of monoamines is illustrated by α -methyl-DOPA which is known to have mildly sedative and blood-pressure reducing properties. This substance is metabolized *in vivo* to α -methylated catecholamines leading eventually to a long-lasting displacement of noradrenaline by α -methyl-noradrenaline, a substance which is not attacked by monoamine oxidase (CARLSSON and LINDQVIST 1962). Dopamine and 5-hydroxytryptamine are moderately reduced only for a few hours by the α -methylated catechol analogues (SOURKES *et al.* 1961, PLETSCHER and GEY 1962).

4) The monoamine oxidase inhibitors have been shown to act not only on the metabolic break-down of the catecholamines, but also by preventing

the release of the amines from their binding sites in the tissues (AXELROD, HERTTING and PATRICK 1961).

Catabolism: The major metabolic pathway of the monoamines in the mammalian brain seems to be the oxidative deamination by monoamine oxidase (SPECTOR *et al.* 1960, ROSENGREN 1960 b, GEY and PLETSCHER 1961). Catecholamines released into the peripheral blood or administered exogenously are inactivated primarily by the catechol-O-methyltransferase (COMT) of the liver and kidney (AXELROD 1959). This enzyme was shown to occur also in small concentrations in the brain (AXELROD 1958). In a study by CARLSSON and HILLARP (1962) the administration of DOPA to rabbits caused initially an increase in the brain of the monoamine oxidase product, 3,4-dihydroxyphenylacetic acid (DOPAC), which was transformed secondarily to the methoxylated COMT product, homovanillic acid. This finding supports the view that the catecholamines of the brain are primarily attacked by monoamine oxidase.

Administration of monoamine oxidase inhibitors results in an increased brain level of 5-hydroxytryptamine (for which monoamine oxidase is probably the only metabolic pathway). The noradrenaline of the brain increases in some species (rats, dogs), but remains unaffected in others (cats, for instance), following administration of monoamine oxidase inhibitors. When the noradrenaline level is high, there is generally a more or less marked excitement (BRODIE, SPECTOR and SHORE 1959 b). The excitement is increased by administration of DOPA (CARLSSON *et al.* 1957), 5-HTP (UDENFRIEND *et al.* 1957) and by reserpine (SHORE and BRODIE 1957) or the α -methylated catechol analogues (COSTA *et al.* 1962). All these substances produce excitement, probably by increasing the amount of "free" amines at synaptic sites. Whether dopamine is the most important of the catecholamines in this respect cannot be regarded as settled. BERTLER (1960) demonstrated a close correlation in time between the dopamine content of the brain and the signs of excitement after administration of DOPA in rabbits.

For 5-hydroxytryptamine the relation to states of excitement is more obscure. A central tranquilizing action has been ascribed to it (SULSER and BRODIE 1960, GESSA *et al.* 1962).

Effects of morphine on catecholamines and 5-hydroxytryptamine

ELLIOTT (1912) reported an adrenaline-depleting action of morphine in the adrenal glands of cats and dogs. It was noticed that the effect could be prevented by sectioning the splanchnic nerve before the administration of morphine and thus that it was mediated through nervous stimulation.

The observation that morphine reduces the adrenaline content of the adrenals and/or elevates the catecholamine content of the adrenal vein blood, has been confirmed in cats (STEWART and ROGOFF 1922, EMMELIN and STRÖMBLAD 1951, VOGT 1954), rabbits (ABE 1929, TADA 1932), rats (OUTSCHOORN 1952) and dogs (SATO and OHMI 1933, WADA *et al.* 1938, SIBUTA, ENDO and NAGAKURA 1949).

An increase in the adrenaline concentration of peripheral blood after morphine injection was reported in rabbits (HAYAMA 1932) and dogs (RICHARDSON, WOODS and RICHARDSON 1958). The urinary output of catecholamines increased after morphine injection in rats (CRAWFORD and LAW 1958).

ABE (1929) found an increased amount of adrenaline in the adrenal glands of morphine tolerant rabbits and a disappearance of the adrenaline depleting effect after long-term morphine treatment. TACHIKAWA (1932) noticed that in the blood of chronically morphine-treated dogs "there is a greater amount of adrenaline in the non-morphine period". WADA *et al.* (1938) reported that the morphine-induced output of adrenaline in the adrenal vein blood of dogs was diminished as a result of morphine tolerance.

VOGT (1954) reported that, in addition to the catecholamine depletion of the adrenal glands, there was a concomitant reduction of the midbrain and hypothalamic stores of "sympathin" in cats given 30—60 mg/kg of morphine. In dogs there was only a slight corresponding effect. QUINN, BRODIE and SHORE (1958) confirmed this observation in cat brains but found no effect of morphine on the catecholamines of rabbit brains.

5-Hydroxytryptamine was not changed in the rabbit brain after single morphine doses (BRODIE, SHORE and PLETSCHER 1956). Long-term administration of morphine in rats did not influence the brain 5-hydroxytryptamine (COCHIN and AXELROD 1959).

No reports dealing with the effects of long-term morphine administration or withdrawal on the brain catecholamines seem to have appeared before 1959, when my first paper on this subject was published. Some recent reports are discussed later in the present work. — No earlier studies (except my own 1961, 1962 a, b) of the urinary output of catecholamines and 5-HIAA in morphine tolerance and withdrawal are known to the author.

CHAPTER II

GENERAL METHODS

I. Experimental procedures

Experimental animals

Dogs, rats and cats were used in the present investigation. The animals were fed a balanced commercial brand of laboratory food, and were allowed food and tap water *ad libitum*.

In the experiments to be described 45 mongrel *dogs* of either sex were used. The animals ranged in weight from 4 to 15 kg. Approximately the same number of males and females was placed in each experimental group. The dogs were sacrificed by a gun-shot through the heart.

A total of 330 male albino *rats* of the Sprague-Dawley strain (Anticimex, Stockholm) were employed. When possible adult rats, weighing 250—300 g, were used. The weights of the animals in each group did not vary more than ± 10 g at the beginning of an experiment. The rats were sacrificed by decapitation.

Fifteen *cats* of either sex, weighing 2.5—4.0 kg were used in acute and short-term morphine experiments. The cats were sacrificed by decapitation following chloroform anaesthesia.

Administration of morphine and nalorphine

A 4 per cent solution of morphine hydrochloride, rendered isotonic with 0.3 per cent of sodium chloride, was used. For stabilization of the morphine hydrochloride 0.1 per cent of 1 N hydrochloric acid was added to obtain a final pH around 4. This solution is referred to as *morphine* throughout this paper.

Nalorphine was given as a 1 per cent solution of nalorphine hydrochloride (Nalorphin, Leo Ltd.).

Dogs and cats were given subcutaneous injections, while rats received intraperitoneal injections of morphine and nalorphine.

Rats and cats were used in acute morphine experiments. The injections were given at 9 a. m. and the animals were sacrificed four hours later. In a few experiments rats and cats were treated daily with morphine for only a few days. The rats were injected twice daily at 9 a. m. and 5 p. m. and

the cats once daily. These animals were sacrificed four hours after the last injection, given at 9 a. m.

Dogs and rats were given chronic morphine treatment. The initial morphine dose in the *dogs* was 3 mg/kg given once daily at 9. a. m. This dose was increased at intervals of 7 to 10 days until 40 mg/kg were reached after 6 to 8 weeks. After this the dose was divided and given at 9 a. m. and 5 p. m. The morning dose was half the evening dose. During the first week of split doses, 20 mg/kg were given in the morning and 40 mg/kg in the afternoon; next week the doses were 30 and 60 mg/kg, etc. The final dose was 90 to 120 mg/kg/day and the whole series of injections lasted for 70 to 90 days. In a few instances some dogs were readdicted after withdrawal for three weeks. The second cycle of addiction was shortened to about four weeks.

This schedule of administration is referred to as *chronic* morphine treatment throughout this paper. Dogs given chronic morphine treatment are referred to as *morphine tolerant*.

The *rats* were injected for three weeks according to two different dosage regimens:

1. *Standard regimen* (20—180 mg/kg, twice daily).

Injections were given twice daily at 9 a. m. and 5 p. m. starting with 20 mg/kg/injection. The dose was tripled every seventh day and the dose levels of the three weeks were thus: 20, 60 and 180 mg/kg/injection.

2. *Intense regimen* (20—300 mg/kg, at 4 hours' intervals).

During the first week injections were given twice daily at 9 a. m. and 5 p. m. of 20 mg/kg/injection. From the eighth day five daily injections were given at 7 a. m., 11 a. m., 3 p. m., 7 p. m. and 11 p. m. The dose was increased by 20 mg/kg/injection every day (20, 40, 60, 80 . . . mg/kg/injection). After fourteen days the rats were given six daily injections every fourth hour around the clock until, on the twentyfirst day they received 300 mg/kg/injection, corresponding to a daily dose of 1 800 mg/kg.

These two schedules of morphine administration are referred to as *chronic* treatment and rats so treated as *morphine tolerant* rats.

When dogs and rats had been rendered morphine tolerant, the following treatments were employed:

- a. The regular morphine injection at 9 a. m. was substituted by one injection of saline. Dogs were given an additional saline injection at 11 a. m. and the animals were sacrificed four hours after the first saline injection.
- b. The regular morphine injection at 9 a. m. was substituted by nalor-

phine. Dogs were given two consecutive injections of 5 + 5 mg/kg at 9 a. m. and 11 a. m., while rats received one injection of 10 mg/kg. The animals were sacrificed four hours after the (first) nalorphine injection.

c. The animals were withdrawn from the regular morphine injections, dogs for 72 hours and rats for 48 hours, before sacrifice.

One group of *control dogs* was untreated. Another control group received two consecutive injections of nalorphine 5 + 5 mg/kg at two hours intervals and was sacrificed four hours after the first injection. The *control rats* were given saline in amounts corresponding to the morphine treated animals. One group of control rats received in addition a single injection of 10 mg/kg of nalorphine and was sacrificed four hours later.

When urine was collected from four morphine tolerant dogs the animals were stabilized on single daily morphine injections, 90 mg/kg, given at 9 a. m. This morphine dose was substituted by one injection of nalorphine, 10 mg/kg, whereafter the morphine administration was resumed.

Collection of urine

When dogs and rats were kept in metabolic cages, special precautions had to be taken to avoid fragmentation of food into small particles, which might absorb considerable amounts of urine. During these periods the dogs were fed only meat, while the rats' diet was ground and thoroughly mixed with water.

Young *dogs*, weighing 3—5 kg, were housed in individual metabolic cages. The floor consisted of cylindrical plexiglass rods, 10 mm in diameter inserted at 5 mm distances from each other. The urine was collected in glass flasks through plexiglass funnels in which a plug of pyrex-wool was placed, to filter out fecal solids.

Fifty ml of 1 N sulphuric acid were usually added to the bottles every 24 hours to produce an approximate pH of 3. The cages were cleaned daily for ½ hour, during which time the animals were allowed to move freely. For convenience the 23½ hours urine was calculated as 24 hours urine.

Since no special measures were observed to avoid leakage of drinking water directly into the funnels, the values for the urine volumes may have been too high. However, together with the salivation of the dogs, the additional volume probably amounted only to a few per cent. When vomiting occurred, as in one dog during nalorphine-induced abstinence, this animal was excluded when the mean urine volumes were calculated. — Naturally the calculation of the urinary amines was uninfluenced by these sources of error.

Five rats were placed in each metabolic community cage. There were no metal parts in the collecting system. The floor, 50 cm in diameter, was made of perforated plexiglass and placed on a glass funnel with arrangements for separation of fecal solids. Ten ml of 1 N sulphuric acid were added, as a preservative, to the polythene collecting bottles.

2. Estimation of catecholamines

Noradrenaline and adrenaline were estimated in the brains and adrenals of dogs, cats and rats and the urine of dogs and rats. Dopamine was estimated in the brains of dogs.

Choice of method

For determination of catecholamines in the brains of dogs and cats a modification of the method of EULER and LISHAJKO (1961) was used. A preliminary study (*cf.* below) showed that the results with this convenient method were almost identical with those obtained by the somewhat more time-consuming method of BERTLER, CARLSSON and ROSENGREN (1958).

Estimation of catecholamines in the rat brain was made by the method of BERTLER *et al.* (1958) for the following reasons. — The experiments with rats included administration to the animals of α -methyldihydroxy-phenylalanine (α -methyl-DOPA) a substance which is converted in the brain to α -methylated catecholamines (CARLSSON and LINDQVIST 1962). Alpha-methyl-dopamine, when treated according to the trihydroxyindole (THI) method, gives a strongly fluorescent product, which would invalidate the estimation of noradrenaline. Therefore it was thought advisable to exclude interference from α -methyl-dopamine by the chromatographic separation on Dowex 50. The fluorophore of α -methyl-noradrenaline, on the other hand, which is not separated from noradrenaline by this method, had a weak fluorescence, less than 5 per cent of that of noradrenaline with the filter sets used in the present investigation. This is in accordance with earlier reports (LUND 1949, CARLSSON and LINDQVIST 1962).

Dopamine was estimated in the telencephalon of dogs according to CARLSSON and WALDECK (1958).

The extracts of adrenals were diluted with sodium acetate buffer solution and the determination of adrenaline and noradrenaline was then performed as for brain and urinary eluates.

Free urinary adrenaline and noradrenaline were determined by the method of EULER and LISHAJKO (1961).

A. Extraction

Extraction of brain tissue was performed with 0.4 N perchloric acid. Only one extraction was made and the final catecholamine values were calculated according to BERTLER *et al.* (1958), presuming a water content of the tissue of 75 per cent. This implies the following correction factor for the catecholamine values found:

$$(\text{Extraction vol.} + 75 \% \text{ of wet tissue wt.})/(\text{vol. taken for assay})$$

The *dog brain* was dissected out immediately after sacrifice, stripped of the meninges, and divided into three parts: 1) telencephalon, 2) cerebellum and 3) brain stem (medulla oblongata, pons, midbrain and diencephalon, but excluding the basal ganglia belonging to the telencephalon). Each part was weighed and homogenized (Ultra-Turrax homogenizer) in six volumes of cold 0.4 N perchloric acid. The extraction was allowed to proceed for about 30 min. at $+4^{\circ}\text{C}$ after homogenization. After centrifugation the supernatant was passed through a filter paper (Munktell, density grade 00). Generally 40 ml of the extracts of telencephalon and cerebellum, and 15 ml of the brain stem extracts were taken for purification and assay.

The whole *rat brains* were dissected out and put in 0.4 N perchloric acid. Two brains were pooled for each determination, homogenized and extracted in 20 ml (six volumes) of cold acid. The extracts were treated as described above for dog brains. All extracts were stored at -30°C for not more than one week. The whole extract was taken for assay.

The *cat brain* was removed and the brain stem (here defined as medulla oblongata, pons, midbrain and diencephalon) was cut out. It was extracted with six volumes of 0.4 N perchloric acid and treated as described for the dog brains.

The *adrenals* of dogs, rats and cats were dissected out 15 to 30 min. after death. The organs were carefully freed from adherent tissue, weighed on a torsion balance and ground in a mortar with quartz sand, together with 5 per cent trichloroacetic acid. Adrenals of dogs (one pair) and cats (one pair) were extracted in 50 ml. For the rats' adrenals (two pairs) 10 ml were used. After filtration the extracts were generally stored at -30°C for 1 to 7 days before further dilution and fluorimetric estimation.

Stability of noradrenaline in perchloric acid extracts

The pH of the perchloric acid brain extracts was about 0.5. The studies

of WEST (1952) indicate, however, an optimal stability for catecholamines at pH 3.5 to 3.9. In order to study the stability of noradrenaline in the perchloric acid extracts stored at -30°C , the following experiment was undertaken.

The brain stems of three dogs, with a total tissue weight of 25 g were extracted with 150 ml of 0.4 N perchloric acid. The pooled extract was divided in ten parts of 15 ml each. Two samples were determined on the day of extraction to obtain reference values. Four samples were adjusted to pH 3.8 with 2 N potassium hydroxide, and together with the four extracts at pH 0.5 they were stored at -30°C . At intervals of one month two samples were taken out, one at pH 3.8 and one at pH 0.5 and determined as above.

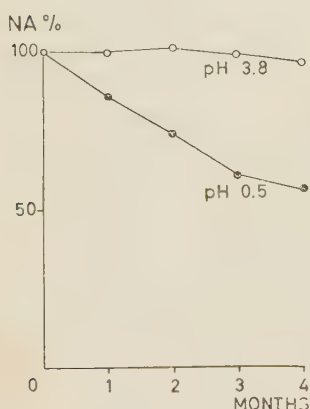


Fig. 1. Stability of noradrenaline (NA) in 0.4 N perchloric acid extract of dog brains (pH 0.5). Part of the pooled extract was adjusted to pH 3.8 before storage at -30°C .

The results are given in Fig. 1 from which it is evident that 3.8 is a more favourable pH for storage. At pH 0.5 the noradrenaline content was reduced to about 50 per cent in four months. The gradual reduction of noradrenaline was obviously not prevented by storage at -30°C .

In accordance with BERTLER *et al.* (1958), it was found that the samples could be stored for a week without appreciable loss. If the time for storage was extended beyond a week, the samples were adjusted to pH 3.8.

B. Purification

As mentioned previously, the brain extracts were purified according to two different methods. These will be briefly described below:

Method of BERTLER, CARLSSON and ROSENGREN (1958):

A column, 4.2 mm in diameter, containing 300 mg of the strongly acid cation exchange resin Dowex 50 W X4, 200—400 mesh, Na⁺ form, was used. The resin had been washed with 5 N hydrochloric acid, glass-distilled water and 1 N sodium hydroxide. Forty ml of 2 N HCl, redistilled water, and 1 N sodium acetate buffer, pH 6.0, were passed through the column, prior to analysis.

The perchloric acid extracts were thawed and adjusted to pH 4 with 2 N potassium hydroxide. A pH-meter (Metrohm) and a magnetic stirrer were used in all titrations. The precipitate of potassium perchlorate was spun down at 0° C, and the extract was passed through the columns.

After two washings with glass-distilled water, elution was performed with 1 N hydrochloric acid in two separate portions: the first 9 ml of the eluate contained noradrenaline and adrenaline. The second portion of the eluate (10 ml) contained approximately 90 per cent of the dopamine content of the sample.

The same column could be used again after rinsing with 2 N hydrochloric acid and regeneration as described in the original method.

Method of EULER and LISHAJKO (1959 and 1961):

The perchloric acid extracts were thawed, adjusted to pH 4 with 2 N potassium hydroxide and the perchlorate was spun down. The pH of the supernatant was adjusted to 8.4 with 0.1 N sodium hydroxide and the extract was passed through a column 0.9 cm in diameter containing 1 g of aluminium oxide (British Drug Houses), previously washed with glass-distilled water to let fine particles pass through the glass filter. After adsorption the column was carefully washed with glass-distilled water and elution was performed with 0.25 N acetic acid.

When the extracts were clear and colorless (as for instance dog brain stem and cerebellum extracts) the potassium hydroxide titration and centrifugation steps could be omitted and pH adjusted to 8.4 directly by 1 N sodium hydroxide.

Adrenal extracts could be assayed for catecholamines after dilution without purification. The extract was thawed and thoroughly stirred in order to avoid the separation brought about by freezing (the "cryoscopic effect", RANGAPPA 1961). For *dogs'* adrenals 0.1 ml of the extract was generally diluted in 9.9 ml 0.15 N sodium acetate buffer at pH 6.5. For the *rats'* and *cats'* adrenals 0.5 ml of extracts was diluted in 9.5 ml 1 N sodium acetate buffer at pH 6.8. After dilution the final pH before assay was 6.3 in all extracts.

The *urine* of dogs and rats was filtered, whereafter 25 ml or less were treated according to the method of EULER and LISHAJKO (1959), including addition to the urine of the disodium salt of ethylenediaminetetraacetic acid (EDTA).

Addition of EDTA

When the disodium salt of ethylenediaminetetraacetic acid (EDTA; Titriplex, Merck) was added to the brain extracts, in amounts corresponding to those recommended for the same volumes of urine (about 140 mg/g of wet tissue), the noradrenaline values were considerably depressed. In order to secure the relationship between the lowered values and the addition of EDTA the following study was undertaken:

The pooled extract of ten rat brains in 100 ml 0.4 N perchloric acid was divided in five equal samples to which various additions of EDTA were made. The samples were purified on alumina and assayed according to EULER and LISHAJKO (1961). It was found that even small amounts of EDTA lowered the noradrenaline values (Table I). Addition of EDTA

Table I. Influence of added amounts of the disodium salt of ethylenediaminetetraacetic acid (EDTA) on the noradrenaline (NA) content in rat brains. Extracts of 10 rat brains pooled and divided in 5 equal samples

EDTA mg/g	NA %
0	100
5	70
10	68
15	50
50	50

was for this reason omitted for brain extracts and was found unnecessary when the extraction volume was sufficiently large to prevent precipitation (of phosphates and other salts) at pH 8.

When treating the urine samples, EDTA could not be dispensed with, unless the urine was either diluted to avoid precipitating salts, or centrifuged in order to remove the precipitate at pH 8.4. These steps lowered the recovery yields and were abandoned.

Comparison between the methods of EULER and LISHAJKO (1961) and BERTLER, CARLSSON and ROSENGREN (1958)

As two different methods of purification of the brain extracts were used, the following studies were undertaken to evaluate the accuracy and specificity of these methods:

Table II. Comparison between purification on Alumina and Dowex 50 of a solution of noradrenaline (NA) 2 μ g/10 ml in perchloric acid

	Alumina NA μ g/10 ml	Dowex 50 NA μ g/10 ml
	1.68	1.70
	1.69	1.74
	1.73	1.76
	1.78	1.76
	1.78	1.82
Means	1.73	1.76
Recovery	87 %	88 %

Standard solutions: To a solution of 0.4 N perchloric acid, noradrenaline hydrochloride was added, 2 μ g/10 ml, and potassium chloride corresponding to the approximate ion concentration of a brain extract. Five samples of 15 ml of this simulated brain extract were adjusted to pH 4 with a measured volume of 2 N KOH, centrifuged and a 10 ml aliquot of the extract was passed through a column of Dowex 50. (This procedure eliminates the losses normally included in the centrifugation step). Five other samples were adjusted to pH 8.4 by 1 N sodium hydroxide and passed through columns of alumina. The eluates were assayed fluorimetrically as described below.

The values, expressed as μ g/10 ml of the "extract", show that both methods gave almost identical results (Table II).

Table III. Comparison between purification on Alumina and Dowex 50 of noradrenaline (NA) in brain stem extracts of dogs. The pooled extract of untreated dogs divided into 10 equal samples

	Alumina NA μ g/g	Dowex 50 NA μ g/g
	0.35	0.32
	0.35	0.37
	0.36	0.33
	0.36	0.34
	0.35	0.32
Means	0.35	0.33

Table IV. Comparison between purification on Alumina and Dowex 50 of noradrenaline (NA) in brain stem extracts of dogs. The dogs had been given chronic morphine (Mo) treatment, final dose 100 mg/kg. Three dogs received 5 + 5 mg/kg of nalorphine before sacrifice

Treatment	Alumina NA μ g/g	Dowex NA μ g/g	Difference
Chronic Mo	0.34	0.35	- 0.01
"	0.30	0.30	0
"	0.32	0.30	+ 0.02
Chronic Mo + Nalorphine	0.24	0.21	+ 0.03
"	0.12	0.13	- 0.01
"	0.25	0.22	+ 0.03

Brain extracts of untreated dogs: Two hundred ml of pooled extract from brain stems of untreated dogs were divided in ten samples of 20 ml each. Five of the extracts were treated by the method of BERTLER *et al.* (1958) and five according to EULER and LISHAJKO (1961). The results with both methods were practically the same (Table III).

Brain extracts of chronically morphine-treated dogs: The brain stem of three chronically morphine-treated dogs were extracted with 0.4 N perchloric acid. From each extract two 15 ml samples were taken for purification and determination by each method. The same experiment was repeated in three other chronically morphine-treated dogs who had received in addition two injections of nalorphine, 5 mg/kg, four and two hours before examination. In spite of the different treatments there was still a satisfactory agreement in the results obtained by both methods (Table IV).

Addition of morphine to brain extract: The pooled extract of eight rat brains was divided in four equal parts. To two of these, morphine hydrochloride was added in amounts corresponding to 40 μ g/g wet tissue and the extracts were treated by the method of BERTLER *et al.* (1958). Other workers (JOHANNESON 1962 and MILTHERS 1962) have shown that higher doses of morphine than those used in the present investigation, produce brain levels approximating this concentration.

The addition of morphine HCl did not influence the catecholamine

levels, obtained after purification on Dowex 50. This is in accordance with earlier results (GUNNE 1961), when morphine was added to urine and the purification was made on alumina.

Stability of eluates: Since the pH of the hydrochloric acid eluates of the method of BERTLER *et al.* (1958) was found to approximate 0.5 the samples were probably not stable for any length of time (*cf.* Fig. 1). For this reason the final assay was made without delay on the day of elution.

The acetic acid eluates of the method of EULER and LISIAJKO (1961) had a pH around 3.5 and could be stored in the cold for months without appreciable loss.

These studies indicate that, for the purpose of the present investigation, the two methods of purification gave results which were comparable with regard to accuracy and specificity.

C. Assay

The assay of adrenaline and noradrenaline in the final eluates was performed by fluorimetric technique, using the trihydroxyindole (THI) method (EHRLÉN 1948, LUND 1949, 1950, EULER and FLODING 1955). This includes oxidation of the catecholamines and rearrangement of the oxidized products in alkaline solution to the corresponding strongly fluorescent lutines, using ascorbic acid as a stabilizing agent. Blanks were prepared either by omitting the ascorbic acid until the fluorescence had disappeared (faded blank) or by omitting the oxidation step (non-oxidized blank). Though both methods for preparing the blank gave comparable results, the faded blanks were found to be more stable and were chosen for this reason for all eluates. The non-oxidized blanks were used only for analysis of adrenal extracts which presumably contain very little interfering material.

The final determination of noradrenaline and adrenaline was made on a Coleman fluorimeter type 12 C, using two sets of monochromatic filters as described by COHEN and GOLDENBERG (1957). The filter set *a* consisted of a primary monochromatic filter with a peak transmission at 395 nm (the filter was a combination of: Schott UG 3, BG 14, CG 13) and as a secondary filter Ilford Bright 623 with peak transmission at 490 nm. The filter set *b* consisted of a primary monochromatic filter with peak transmission at 436 nm (the filter was a combination of Schott BG 12, GG 15) and as secondary filter Corning 3486 with peak transmission 540 nm.

A plexi-glass rod was used as standard, which was repeatedly calibrated

against solutions containing the fluorophores of noradrenaline and adrenaline. A recalibration was required every 2 to 3 weeks.

Using these filter sets the fluorescence of noradrenaline is approximately equal with *a* and *b* filters, while the fluorescence of adrenaline is about three times higher with *b* filters compared with *a* filters.

A slow gradual change was noticed in the sensitivity of the fluorimeter, probably as a result of changes in the transmission qualities of the filters. It was found that this gradual change followed a pattern, which was to a certain extent predictable. It implied an inherent tendency of the instrument to obtain an increased sensitivity to adrenaline. If the plexi-glass standard was not checked against catecholamine standards for some interval, the values were biased in the direction of too high adrenaline figures, while the noradrenaline values were still approximately correct.

Dopamine was estimated according to CARLSSON and WALDECK (1958). The fluorescence was read in a spectrophotofluorimeter (Aminco-Bowman) at the peak fluorescence wavelengths and compared with known standard solutions.

Procedure

The hydrochloric acid eluates were titrated to pH 6.0—6.5 with 2 N potassium hydroxide, after addition of 0.1 ml 5 N acetic acid to obtain an increased buffer capacity. (Titration with potassium carbonate, as recommended by BERTLER *et al.* (1958), was not used since it was found difficult to obtain a stable pH in spite of rapid stirring with a magnetic stirrer). The acetic acid eluates were titrated with 1 N ammonium hydroxide.

Adrenaline and noradrenaline: 3 ml of the neutralized brain eluates were taken for each determination. The eluates were oxidized by the addition of 0.1 ml of 0.25 per cent potassium ferricyanide solution. Exactly three min. later 3 ml of a mixture containing 5 N NaOH (88 %), a 2 per cent solution of ascorbic acid in water (10 %) and concentrated ethylene diamine (2 %), were added to the oxidized eluate. The sample was then diluted to 10 ml with water. Blanks were prepared by omitting the ascorbic acid, which was added 5 min. later.

In the analysis of urine samples 3 ml of neutralized eluate were used to 4 ml of alkaline-ascorbate-EDA mixture. For extracts of adrenals 1 ml of the diluted extract pH 6.3 was used with 1 ml of the mixture. This mixture remains stable for 3 to 4 hours (EULER and LISHAJKO 1961).

The noradrenaline and adrenaline values were calculated by solving the equations:

$$m = yN_a + xA_a$$

$$n = yN_b + xA_b$$

In these equations x and y represent the amounts of adrenaline and noradrenaline respectively. m and n were the fluorimetric readings (corrected for blanks) for filter sets a and b respectively. A_a , A_b , N_a and N_b were the values for the fluorescence per μg on filter sets a and b for adrenaline and noradrenaline respectively.

Dopamine: The determination of dopamine was made according to CARLSSON and WALDECK (1958). Some of these investigations were carried out on the second eluate from columns of Dowex 50 containing mainly dopamine. The presence of noradrenaline in eluates from alumina did not interfere. This is in agreement with CARLSSON and WALDECK (1958) and LEDUC (1961).

Adrenaline in the brain

The relative adrenaline content of mammalian brains is small and its presence has even been questioned. This problem was the subject of a special study (GUNNE 1962 c). A total chromatographic separation of adrenaline from the other catecholamines was undertaken by the method of KIRSHNER and GOODALL (1957) using the weak cation exchange resin Amberlite IRC 50. The considerable losses in the procedure were compensated for by pooling a large number of brains. In spite of this, 4 per cent of adrenaline (of the sum of noradrenaline and adrenaline) was the lowest detectable amount. Adrenaline concentrations above 4 per cent were found in brains of rats (4.5 per cent) and pigs (7.5 per cent). Later an improved separation technique using the strongly acid cation exchange resin Amberlite CG 120 (HÄGGENDAL 1962) was adopted. The use of this resin permitted the detection of even smaller amounts of adrenaline. The adrenaline concentration in dog brains was found to approximate 2 to 3 per cent of the sum of adrenaline and noradrenaline (unpublished results).

It was noticed when brain eluates were assayed on the Coleman fluorimeter, that negative adrenaline values were often obtained in eluates from the telencephalon of dogs. This was probably due to some interfering substance in the brains.

By separation of brain eluates from dogs on Amberlite CG 120 a fraction was obtained which gave higher readings with the a filters than with the b filters (samples treated by the THI method). A substance with those properties might obscure the presence of small amounts of adrenaline.

Considering all the implications involved in the estimation of the small

amounts of adrenaline in brain tissue, it was not thought feasible to study changes in brain adrenaline in the present investigation.

3. Estimation of 5-hydroxytryptamine and 5-hydroxy-indoleacetic acid (5-HIAA)

5-hydroxytryptamine was estimated in the brain stem of dogs and total brain of rats. 5-HIAA was estimated in the urine of dogs and rats.

The brain content of 5-hydroxytryptamine was determined according to a method described by BERTLER (1961). Extraction was made with six volumes of 0.4 N perchloric acid. After homogenization the extract was centrifuged and filtered. 10 ml of the extract of dog brain stem were taken for purification and assay. The total extract (approximately 10 ml) of single rat brains were treated the same way.

The method of purification and assay is briefly presented here:

Method of BERTLER (1961)

The extract was purified on a column 6×25 mm of the weak cation exchange resin Amberlite XE-64. After decantation of the smallest particles the columns were prepared and washed with 2 N hydrochloric acid, redistilled water, 20 ml of N sodium acetate buffer at pH 6.5 and finally a few ml of redistilled water.

The extract was adjusted to pH 6.5 by 2 N potassium hydroxide and the perchlorate spun down at 0° C. The sample was then passed through the column. After washing with 10 ml of a 0.02 M phosphate buffer pH 6.5, containing 0.2 % EDTA followed by a few ml of redistilled water, elution was performed by 3 ml of 1.2 N hydrochloric acid. Concentrated hydrochloric acid (0.8 ml) was added to the eluate and the fluorescence was read in an Aminco-Bowman spectrophotofluorimeter at 295 nm activating and 550 nm fluorescence wavelengths.

5-HIAA in urine was determined according to UDENFRIEND, WEISSBACH and CLARK (1955) with the modification of MCFARLANE *et al.* (1956).

Stability of 5-hydroxytryptamine in brain extracts

BERTLER (1961) had noticed that the immediate centrifugation of the homogenate was required in order to preclude losses of 5-hydroxytryptamine. It was suggested that the amine might be adsorbed to insoluble tissue residues. However, in the present study it was found that 5-hydroxytryptamine was lost whether the extract had been centrifuged, or not.

The influence of pH on the stability of 5-hydroxytryptamine in perchloric acid extracts, was studied in the following way: Nine rat brains were pooled and extracted with 90 ml 0.4 N perchloric acid (six volumes). The extract was homogenized immediately, centrifuged and filtered. The resulting pH was approximately 0.5. The extract was then divided into nine equal samples, four of which were adjusted to pH 4 by 2 N KOH and the perchlorate was spun down. One sample was assayed immediately (starting level). Eight samples (three at pH 4 and five at pH 0.5) were stored at -30°C . The samples were taken out at different time intervals, thawed and assayed for 5-hydroxytryptamine (Fig. 2).

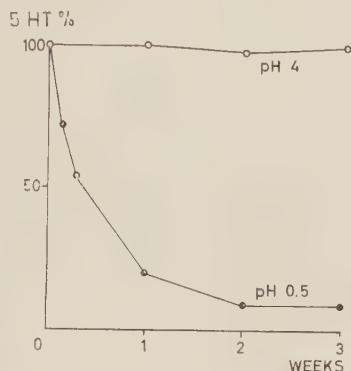


Fig. 2. Stability of 5-hydroxytryptamine (5-HT) in 0.4 N perchloric acid extract of rat brains (pH 0.5). Part of the pooled extract was adjusted to pH 4 before storage at -30°C .

Results. From the figure it is evident that the extracts can not be stored in the strongly acid extraction fluid without considerable losses. An approximate reduction of 50 per cent was found after two days. 5-Hydroxytryptamine was found to be stable at pH 4 (*cf.* The Merck Index Ed. VII. p. 932). This study shows that the rapid loss at pH 0.5 can not be prevented by freezing.

For this reason all extracts intended for 5-hydroxytryptamine determination were adjusted to pH 4 by 2 N KOH before storage.

Modification introduced in estimation of catecholamines and 5-hydroxytryptamine in brain extracts

In summarizing the data presented above, the following modifications were introduced:

Extraction was performed, according to BERTLER, *et al.* (1958), with 0.4 N perchloric acid. Immediately after centrifugation and filtration of the brain homogenate, the pH of the extract was adjusted to 3.5–4.0 in

order to prevent a rapid loss of 5-hydroxytryptamine. The catecholamines were also destroyed in the perchloric acid, but at a slower rate. Adjustment of pH to 3.8 was found necessary, if the extract were to be stored for more than a week.

When purification of the brain extracts was made according to EULER and LISHAJKO (1961), the addition of EDTA (Titriplex) was omitted. This could be done, if care was taken to use sufficiently large extraction volumes (six volumes). Otherwise a flocculation occurred at a pH of 8.

Recovery experiments

The methods were tested repeatedly by adding known amounts of amines to portions of a pooled extract or urine sample. The extracts were usually divided in eight equal parts. Two aliquots were analyzed without the addition of exogenous amines. To the remaining six were added varying amounts of noradrenaline, adrenaline, dopamine, 5-hydroxytryptamine or 5-hydroxyindoleacetic acid. The pooled extracts of brain stem and telencephalon of dogs, adrenals of dogs and rats, total brain of rats and urine of rats and dogs were treated this way. The results are summarized in Table V. The recovery experiments gave satisfactory yields for all substances studied.

The values obtained in the present paper have not been corrected for procedural losses.

Calculation of values and statistical treatment

The catecholamine values given in the present paper are expressed as hydrochlorides. 5-Hydroxytryptamine and 5-HIAA were calculated as free base and acid.

The tissue contents were generally calculated as the amount ($\mu\text{g} = 10^{-6} \text{ g}$ or $\text{ng} = 10^{-9} \text{ g}$) per gram of wet tissue weight. Thus the content of amines in the brains of dogs, rats and cats, and adrenals of dogs and cats was expressed this way, since no significant fluctuations in tissue weights were noted from any treatment employed.

The urinary output in dogs was expressed as $\mu\text{g/kg/h}$ (there were only small variations in the body weight of dogs during the period of observation and no decrease was seen following withdrawal).

In rats the weights of the adrenals were almost doubled following 3 weeks of morphine treatment (adrenal cortex hypertrophy, TANABE and CAFRUNY 1958). The body weight of rats showed great fluctuations during tolerance and withdrawal. For these reasons the catecholamine content of

Table V. Recovery. Pooled extracts or urine was divided into 8 equal parts. Known amounts of noradrenaline (NA), adrenaline (A), dopamine (DA), 5-hydroxytryptamine (5-HT) or 5-hydroxyindoleacetic acid (5-HIAA) were added to six samples in each experiment

Tissue (or urine)	Volume of extract or urine ml	Substance added	Amount added $\mu\text{g/sample}$	Per cent recovered Mean	Per cent recovered Range
Dog telencephalon	40	NA	1—2	98	89—109
	40	A	1—2	76	72— 80
	40	DA	1—2	80	75— 90
Dog brain stem	15	NA	1	83	77— 92
	15	A	0.5—1	70	63— 75
	10	5HT	1	88	83— 91
Rat brain	20	NA	1—2	89	83— 97
	20	A	0.5—2	77	76— 87
	20	DA	1—2	86	82— 92
Dog urine	25	NA	1—2	71	64— 76
	25	A	1—2	68	62— 74
	5	5HIAA	50—100	101	86—120
Rat urine	25	NA	1—2	82	75— 85
	25	A	0.5—2	65	55— 71
Rat adrenals	0.5	NA	0.5—1	98	93—105
	0.5	A	0.2—0.5	99	94—105

the adrenals was expressed as $\mu\text{g}/\text{rat}$ and the amounts excreted in the urine as $\mu\text{g}/\text{rat}/\text{h}$.

Mean, standard deviation (S. D.) and standard error of the mean (S. E. M.) were computed according to ordinary formulae (FISHER 1936)

Significance of means and differences were tested by the t-test (FISHER 1936). Levels of significance:

5 per cent level $P = 0.05—0.01$; probably significant*

1 per cent level $P = 0.01—0.001$; significant**

0.1 per cent level $P < 0.001$; highly significant***

CHAPTER III

GENERAL BEHAVIOR OF DOGS, RATS AND CATS IN MORPHINE TOLERANCE AND WITHDRAWAL

1. General observations

Acute effects of morphine

In *dogs* administration of 3 mg/kg of morphine generally induced sedation or sleep from which the animals could be briefly aroused. The sedation was occasionally preceded by some degree of motor unrest in combination with vomiting. The sleeping time after the first morphine injection sometimes amounted to six hours, but signs of sedation could generally be observed for twelve to twenty-four hours. No postnarcotic excitement was seen in this species.

When *rats* were given a single injection of 20 mg/kg of morphine, there was an initial "sedation" consisting of a more or less abolished spontaneous activity. The condition resembled catalepsy with an increased muscular tone and tendency to remain in peculiar postures. A partial Straub phenomenon was present, the tail was lifted and rigidly extended. This stage was succeeded by one of increased activity. Five to six hours after the injection no further effect could be observed in the rats.

Cats given 5 to 30 mg/kg of morphine were always excited, with dilated pupils and a greatly increased, rather incoordinated activity. The animals were restlessly moving, jumping, rolling on the floor, etc. In some cases general convulsions occurred, sometimes fatal. Some degree of unrest was generally noted for more than twelve hours and was occasionally present even twenty-four hours after the administration of morphine.

Long-term effects of morphine

In *dogs* repeated administration of morphine once daily was followed by a gradually diminished and shortened sedative effect. When the dose was raised there was an increased sedation, but three weeks after the start of injections, the drug did not produce sleep in spite of any increment in dosage. A complete tolerance to the emetic action was obtained within ten

days. After this no vomiting was noticed as long as morphine was given. Salivation was a common finding during the initial two to four weeks of morphine administration. It disappeared in some dogs, only to return in connection with abstinence. After one to two months of morphine administration some dogs became irritable and fought frequently. This was regarded as a sign of physical dependence, since it occurred only at the end of the twenty-four hours between injections and disappeared when the interval was shortened or the dose was raised. Throughout the experiments morphine had a sedative action in dogs and no excitement was seen from the injections.

The *rats* were given morphine according to two different dosage schedules (p. 14) producing different behavioral effects.

The regular dosage regimen, with morphine injections twice daily, resulted in a successively shorter stage of sedation after each injection and an increasingly dominant stage of excitement. After a week on a constant dose, every injection was followed by excitement, with increased activity, hyperpyrexia and exophthalmos. When the dose was increased, stepwise, the signs of sedation reappeared during the first few days of the new dose. The same pattern of increasing excitement was then repeated.

The intense dosage regimen, with morphine injections at four-hour intervals, modified the behavior of the rats. The signs of excitation were not as obvious as with the regular dosage regimen. The rats appeared normal, but occasional outbursts of increased activity were seen. The majority of the rats gained weight and appeared healthy, but sudden deaths occurred from convulsions.

Cats were given morphine for five days. No tolerance to the exciting effect was noticed during these days.

Withdrawal and nalorphine-induced abstinence

When the chronic morphine administration was withdrawn, or the regular morphine injections were substituted by nalorphine, a more or less pronounced abstinence excitation was noticed in *dogs*. The maximal symptoms were seen approximately three days after withdrawal and all signs of abstinence had disappeared after eight to ten days. Following nalorphine administration to morphine tolerant dogs there was a short-lasting state of excitement with a maximal intensity of symptoms one hour after the injection. After another hour the symptoms had usually disappeared. The pattern of symptoms is described in detail below.

In *rats* the withdrawal or nalorphine reaction was not a purely excitatory one. The condition observed after abrupt withdrawal was characterized

by a combination of drowsiness with eyelid ptosis and reduced muscular tone and irritability with hyperalgesia. The animals were difficult to handle, shrieking when lifted by the tail and often trying to bite. During the daytime they were lying rather immobile with their eyes closed, but during the night they fought frequently. Piloerection, tremor and diarrhea were present and the animals lost weight. The symptoms were most marked on the second day of withdrawal and disappeared within eight to ten days. Nalorphine induced corresponding signs lasting for two to three hours. Following the intense dosage regimen the abstinence signs became more profuse, but the general pattern of symptoms was the same and no clearcut excitation was noticed.

2. Tolerance studies in rats

Spontaneous running activity

Methods: The activity cages of DEREK and WANG (1926) were used. These consist of a small living compartment, where the animals can rest, and a revolving drum by which the spontaneous running activity is recorded.

After an acclimation period of two weeks on saline injections, five rats were given chronic morphine treatment according to the standard regimen (20—180 mg/kg twice daily). The cages were artificially illuminated from

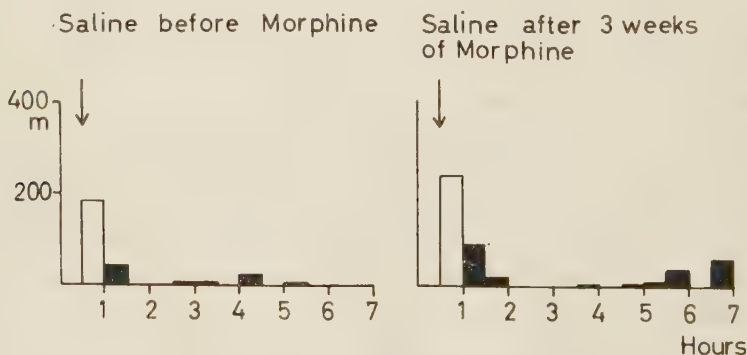


Fig. 3. Spontaneous running activity of rats during light hours, expressed as distance run in meters (mean of five animals). Arrows indicate saline injections. White columns: arousal activity after injections. Black columns: activity during the following six hours. Left figure: activity before morphine. Right figure: activity 24 hours after the last morphine injection.

8 a. m. to 5 p. m. Cleaning of the cages was performed daily between 4 and 5 p. m. The room temperature was between 20° and 22° C.
Results: Fig. 3 illustrates the unspecific arousal activity during the first half hour after the saline injections (white columns), followed by a relatively calm period during the light hours. This pattern of activity was regularly repeated during the control period before the morphine injections were started (left in Fig. 3) and was rapidly resumed after withdrawal of morphine (right in Fig. 3).

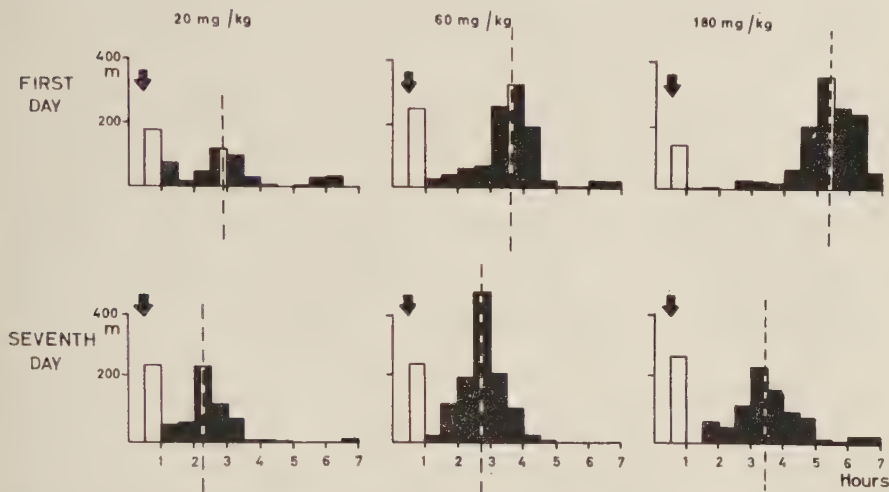


Fig. 4. Spontaneous running activity of rats during chronic morphine administration. For explanation of symbols consult Fig. 3. Doses indicated above. Upper figures: activity on first day of each morphine dose level. Lower figures: activity on the last day of corresponding dose levels. Broken vertical lines: median values of activity (white columns excluded).

During administration of morphine the activity was increased for four to six hours after each injection (Fig. 4). For the purpose of calculation, the first half hour of unspecific injection activity (white column) was excluded and the following six hours were studied. The median values (representing the time when half of the six hours' activity had been produced), were reached later on the first day of a certain dose level (Fig. 4, upper figures), than on the last day of the same dose (Fig. 4, lower figures).

These records show that morphine induced an increase of activity (excitation) in rats and that this activity appeared earlier with increasing tolerance.

Tolerance to toxic doses

The approximate LD_{50} for the rats was 60 mg/kg, which is unusually low. In order to ascertain to what extent the tolerance to toxic doses could be increased, the following investigation was undertaken.

Methods: Ninety-two (male) rats weighing 140—170 g at the start of the experiment were used. Ten were left untreated for three weeks (control animals) while eighty-two rats were made morphine tolerant by the standard dosage regimen (20—180 mg/kg twice daily). On the twenty-first day, with an average weight of 220 g, they received 200 mg/kg in one dose. The controls of an average weight of 240 g, were also given one injection of morphine 200 mg/kg.

Results: The mortality in the controls receiving morphine 200 mg/kg was 80 per cent (eight rats). Of the experimental group 12 per cent (ten rats) died. Nine of these died during the three weeks of pretreatment, prior to the challenging dose of 200 mg/kg (six rats succumbed even to the initial injection of 20 mg/kg).—In later long-term experiments the mortality rate was about the same (10 to 12 per cent) when the final dose was raised to 300 mg/kg.—These experiments show that the rats had acquired a relative tolerance to otherwise toxic morphine doses.

Drug combinations

When control rats received an i. p. injection of the monoamine oxidase inhibiting substance nialamid in a dose of 300 mg/kg they demonstrated drowsiness from which they could be briefly aroused, depression of the righting reflex and eyelid ptosis. This condition lasted for one to two hours and was succeeded by a stage of alertness, increased motor activity and exophthalmos, lasting for more than twenty-four hours.

When the same dose of nialamid was given to morphine tolerant animals four hours after the last morphine injection, the same symptoms appeared. The initial period of somnolence in these animals was, however, considerably longer, generally lasting for three to five hours. After this time the usual alertness appeared.

Following administration of 5 mg/kg i. p. of reserpine there were no noticeable differences between controls and morphine tolerant rats. This drug had a sedative action in both groups of rats.

3. Abstinence signs in dogs

Rating scale

The signs of withdrawal were rated mainly according to PLANT and PIERCE (1928) and grouped into three different grades. The rating scale was well applicable also in nalorphine-induced abstinence.

Grade I Degree of abstinence: SLIGHT

a) *Constant symptoms*

Animals friendly and quiet. Stiff awkward gait, fine tremors, retracted belly.

b) *Common symptoms*

Sleepiness, moderate salivation, diarrhea.

c) *Occasional symptoms*

Vomiting.

Grade II Degree of abstinence: MODERATE

a) *Constant symptoms*

Animals restless, but not continuously moving. Tremors, rigidity with spastic gait. Vomiting. Gnawing at objects.

b) *Common symptoms*

Salivation. Pilo-erection. Twitching of muscles. Diarrhea.

c) *Occasional symptoms*

Whining (generally not noisy). Hiccough. Panting. Growling, but handling possible.

Grade III Degree of abstinence: MARKED

a) *Constant symptoms*

Restless, almost continuously moving. Gnawing and swallowing inedible objects (for instance sawdust). Marked tremors and spastic gait. Pilo-erection. Salivation and vomiting, sometimes profuse.

b) *Common symptoms*

Hiccough, diarrhea. At intervals panting, jerky respiration.

Howling. Muscular weakness.

c) *Occasional symptoms*

Irritable and belligerent. Very noisy.

No dogs in this series showed *very marked* symptoms (grade IV). These were described by PLANT and PIERCE (1928) as "raging wild animals", continuously moving, often with general convulsions.

The ratings were made independently by two observers, generally on the

day of sacrifice and many days before the estimations of monoamines were completed.

Results: Abrupt withdrawal generally gave less severe symptoms than nalorphine. On withdrawal eight out of ten dogs showed slight (grade I), one moderate (grade II), and one marked symptoms (grade III). In twenty-two dogs subjected to nalorphine-induced abstinence seven were grade I, nine were grade II and six were grade III. No convulsions were observed in these experiments.

There did not appear to be any correlation between the addicting dose and the grade of induced abstinence symptoms, and there were great individual variations between dogs treated similarly. Some breeds of dogs (for example beagles) appeared to develop more intense symptoms than others.

4. Discussion

The depressant action of acute morphine administration in dogs and rats, as well as the excitation in cats are in agreement with earlier observations (KRUEGER, EDDY and SUMWALT 1941 p. 8). A difference between the species was found to exist also with regard to the duration of action, four to six hours in rats but twelve to twenty-four hours in dogs and cats.

Chronic morphine treatment produced the well-known tolerance phenomena in dogs and rats, *viz.* a shortening of the duration and a decrease in intensity of the sedative action. In rats, but not in dogs, the depression was converted into a state of excitation. The exciting effect of chronic morphine administration in rats was reflected in the activity cages. Earlier activity studies in rats (TONINI, MISERE and BABBINI 1959 and OGURI 1961) seem to have given comparable results. — No tolerance to the exciting effects of morphine was noticed in the cats, in agreement with the findings of earlier workers using doses within the same range (*cf.* KRUEGER *et al.* 1941, p. 69).

Recently MULÉ and WOODS (1962) reported that the rate of disappearance of free labeled morphine was faster in the brain and blood of morphine tolerant dogs, as compared with nontolerant animals. AXELROD (1956) demonstrated an enzymatic N-demethylating system in liver microsomes of rats, which interacted with morphine during chronic administration. These findings support the view that there is less morphine available at receptor sites in the morphine tolerant organism, but opinions differ concerning their significance for tolerance phenomena (TAKEMORI and MANNERING 1958, HERKEN, NEUBERT and TIMMLER 1959).

The diminished duration of morphine action in dogs, which occurred in a later stage of the addiction cycle, necessitated the introduction of two daily injections. In order to produce dependence on a narcotic drug (and consequently abstinence phenomena) the intervals between injections should not be extended beyond a certain limit (*cf.* REYNOLDS and RANDALL 1957 p. 111). The proper time interval seemed to vary between species, more frequent doses being necessary for rats than for dogs. This might reasonably be attributed to differences in the rate of destruction of the drug.

Abrupt withdrawal and injection of nalorphine produced an abstinence syndrome in dogs and rats (cats were not studied). In dogs abstinence produced a clearcut excitation, while rats exhibited a mixed syndrome (drowsiness and irritability). In both species there were several autonomic manifestations, piloerection, tremor, etc.

It has been pointed out (PLANT and PIERCE 1928, SEEVERS 1936) that a fully developed excitatory syndrome of morphine abstinence with great similarities to that of human addicts can be regularly produced in higher animals like dogs and monkeys.

As far as the abstinence syndrome in rats is concerned, opinions differ. According to some authors (SOLLMAN 1924, MEYERS 1931, KAYMAKALAN and WOODS 1956, MAYNERT and KLINGMAN 1962) the abstinence symptoms in this species are either absent or can be described as a "sedation". Others have noticed signs of irritability and restlessness (JOEL and ETTINGER 1926, BARLOW 1932, HIMMELSBACH, GERLACH and STANTON 1935, DETRICK and THIENES 1941, GUNNE 1961).

Recently a clearcut excitatory abstinence syndrome was reported also in rats (HANNA 1960, WEEKS 1962) and mice (MAGGIOLO and HUIDBORO 1962) when morphine had been administered more or less continuously.

These findings were only partly confirmed in the present study. Following an intense schedule of morphine administration in rats (essentially according to the method of HANNA 1960), the abstinence signs were more pronounced than usual, but not purely excitatory.

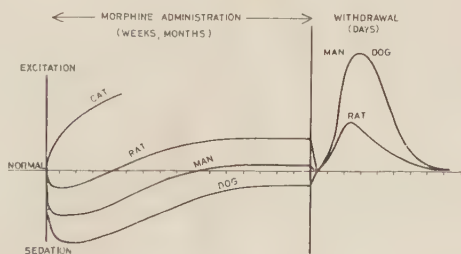


Fig. 5. Schematic representation of the central nervous system excitability in different species during continuous morphine administration with increasing doses (between the vertical lines; time intervals of weeks-months), and after abrupt withdrawal (to the right; time intervals of days).

Fig. 5 is a schematic illustration of the behavior described in different species, during a hypothetical continuous administration of morphine with gradually increasing doses and after abrupt withdrawal. The corresponding curve for man was drawn essentially according to SEEVERS (1954) but a minor modification was introduced, since it was felt that the response to the drug in the morphine tolerant addict is rather one of slight stimulation, than of sedation (unpublished observations). The gradual change of morphine action on the excitability of the central nervous system, thus places man between the dog (which is not excited as long as morphine is given) and the rat (which becomes excited rather soon following the initial sedation). — The excitement produced by morphine administration is complex and in many respects incommensurable with the excitement of withdrawal (ISELL and FRASER 1950), but Fig. 5 may illustrate the approximate species differences also with regard to the withdrawal reaction.

A few morphine tolerance effects were studied separately. It is not generally agreed that rats acquire tolerance to toxic morphine doses. (*cf.* SCHAU-MANN 1957, p. 214). In the rats of the present study there was evidently an increasing tolerance to the toxicity of morphine. Since no tolerance to the convulsive effects can be expected, this probably means that the rat strain used has been especially susceptible to the respiratory depressant action of the drug.

The special tolerance effects noted in the experiment with a monoamine oxidase inhibitor, will be discussed later (p. 75).

CHAPTER IV

NORADRENALINE, DOPAMINE AND 5-HYDROXY- TRYPTAMINE IN THE BRAIN IN MORPHINE TOLERANCE AND WITHDRAWAL

The effects of *acute* morphine administration on the content of noradrenaline in the brain were studied in cats and rats. Dogs and rats were used for studies of *long-term* effects of morphine and *abrupt withdrawal* as well as *nalorphine-induced abstinence*.

1. Special methods

Some long-term effects of morphine on the noradrenaline content of the rat brain were elucidated by administration of drugs interfering with the storage or metabolism of the catecholamines. The drugs were given after completion of a three weeks series of injections with saline or morphine (standard regimen, p. 14) in 112 male rats weighing 250—300 g.

All rats were sacrificed twenty-four hours after the last saline or morphine injection. The rats were divided into four groups, each consisting of 12 to 14 controls and 12 to 18 morphine tolerant rats.

1) The first group (controls and morphine tolerant) was given no additional treatment.

2) The second group (controls and morphine tolerant) was given 300 mg/kg i. p. of the monoamine oxidase inhibitor nialamid (Niamid, Pfizer) four hours after the last regular morphine or saline injection (twenty hours before sacrifice).

3) The third group (controls and morphine tolerant) was given 150 mg/kg i. p. of α -methyl-DOPA (Aldomet, Merck, Sharp & Dohme) four hours after the last regular morphine or saline injection (twenty hours before sacrifice).

4) The fourth group (controls and morphine tolerant) received 5 mg/kg i. p. of reserpine (Serpasil, Ciba) four hours after the last regular morphine or saline injection (twenty hours before sacrifice).

2. Results

Control levels

It was noticed in *rats* of different weight groups that the brain content of noradrenaline was increasing during lifetime. When the noradrenaline content was calculated per gram of wet tissue weight there was still an increase despite the fact that the weight of the brains had also increased as the rats were growing (Fig. 6). This shows that the noradrenaline content of the brain had increased at a higher rate than the tissue weight.

Seasonal variations were not noticed in the brain content of noradrenaline, but care was taken to provide every morphine treated group with a corresponding control group.

The same tendencies were also sometimes noticed in brains of *dogs* of different age; i. e. lower noradrenaline values in young animals. The material was, however, too small to allow definite conclusions.

Acute and five days administration of morphine

Administration of 30 mg/kg of morphine in a single dose reduced the noradrenaline content of the *cat's* brain stem. Three animals died from convulsions within two to three hours after the injection. If these animals were included, there was a 54 per cent reduction ($P < 0.001$) compared with saline injected controls. When the same dose had been repeated for

Table VI. Noradrenaline (NA) $\mu\text{g/g}$ tissue weight, in brain stem of cats following a single (acute) and 5 days administration of morphine (once daily) 30 mg/kg. Individual values represent means of 2 estimations. (For adrenals cf. Table XII)

	Controls NA	Morphine acute ¹ NA	Morphine acute ² NA	Morphine 5 days ² NA
	0.41	0.21	0.23	0.39
	0.48	0.21	0.34	0.50
	0.43	0.12	0.13	0.38
	0.52		0.24	0.39
Means	0.46	0.18*	0.24**	0.42

¹ Died from convulsions 2—3 hours after injection.

² Sacrificed 4 hours after (last) injection.

* Different from controls $P < 0.05$.

** " " " $P < 0.01$.

RAT

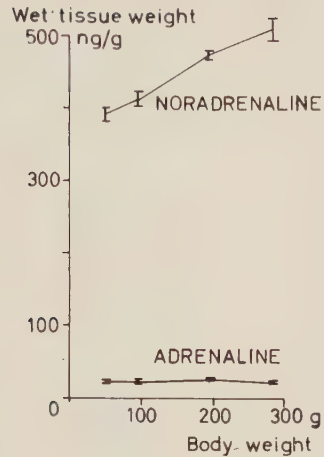


Fig. 6. Adrenaline and noradrenaline content of rat brains, calculated per g of wet tissue weight. Means $\pm$ S.E.M. of groups of twelve rats of different body weights. Two brains pooled for each determination.

five days, the last injection did not cause a significant reduction of noradrenaline (Table VI).

In rats the acute administration of 20 and 30 mg/kg of morphine was without effect. Only when two consecutive injections of 30 + 30 mg/kg were given at two hours intervals was there a reduction of the noradrenaline levels (Table VII).

Table VII. Noradrenaline content in whole brains of rats (means $\pm$ S. E. M.). The rats were given one or two injections of morphine and were sacrificed 4 hours after the first injection. (Second injection was given 2 hours after the first). Two brains were pooled for each determination. (For adrenals cf. Table XIII)

Number of rats	Treatment	Dose	Brain noradrenaline $\mu\text{g/g}$
12	Controls, saline	0.2 ml	0.56 ± 0.03
10	Morphine	20 mg/kg	0.52 ± 0.03
10	"	30 mg/kg	0.52 ± 0.03
10	"	30 + 30 mg/kg	$0.40 \pm 0.03^{**}$

** Different from controls $P < 0.01$.

Table VIII. Noradrenaline (NA) and dopamine (DA) content in dog brain (means $\pm$ S.E.M.). Nalorphine was given 5+5 mg/kg at 2 hours intervals and animals were sacrificed 2 hours after the second injection. Chronic morphine (Mo) treatment was given for 70—90 days, final dose 90—120 mg/kg. Animals sacrificed 20 hours after last injection except in withdrawal group

Number of dogs	Treatment	Total brain NA $\mu\text{g/g}$	Brain stem NA $\mu\text{g/g}$	Cerebellum		Telencephalon	
				NA $\mu\text{g/g}$	NA $\mu\text{g/g}$	NA $\mu\text{g/g}$	DA $\mu\text{g/g}$
10	None	0.16 ± 0.009	0.37 ± 0.015	0.11 ± 0.010	0.13 ± 0.009	0.20 ± 0.013	
6	Nalorphine	0.17 ± 0.014	0.34 ± 0.023	0.11 ± 0.017	0.14 ± 0.011	0.24 ± 0.020	
11	Chronic Mo	0.17 ± 0.008	0.35 ± 0.010	0.12 ± 0.012	0.15 ± 0.009	0.19 ± 0.012	
2	Chronic Mo+ withdrawal 72 h	0.08	0.23	0.03	0.06		0.12
16	Chronic Mo+ nalorphine	$0.10 \pm 0.009^{***}$	$0.21 \pm 0.020^{***}$	$0.06 \pm 0.004^{***}$	$0.08 \pm 0.008^{***}$	$0.13 \pm 0.014^{**}$	

** Different from chronic Mo: $P < 0.01$.

*** Different from chronic Mo: $P < 0.001$.

Table IX. Noradrenaline (NA) content in the whole brains of rats (means $\pm$ S.E.M). Chronic morphine treatment was given for 3 weeks, up to a final dose of 300 mg/kg, given 6 times daily at 4 hours intervals (1 800 mg/kg/day). The rats were killed 4 hours after the last injection of saline or nalorphine with the exception of the withdrawal group. Two brains pooled for each determination (cf. Table XV for adrenals of same rats)

Number of rats	Treatment	NA μ g/g
10	Saline	0.53 $\pm$ 0.012
10	Nalorphine 10 mg/kg	0.56 $\pm$ 0.009
12	Chronic morphine+saline 1 inj.	0.61 $\pm$ 0.015
12	Chronic morphine+nalorphine 10mg/kg	0.60 $\pm$ 0.017
6	Chronic morphine+withdrawal 48 h	0.61 $\pm$ 0.016

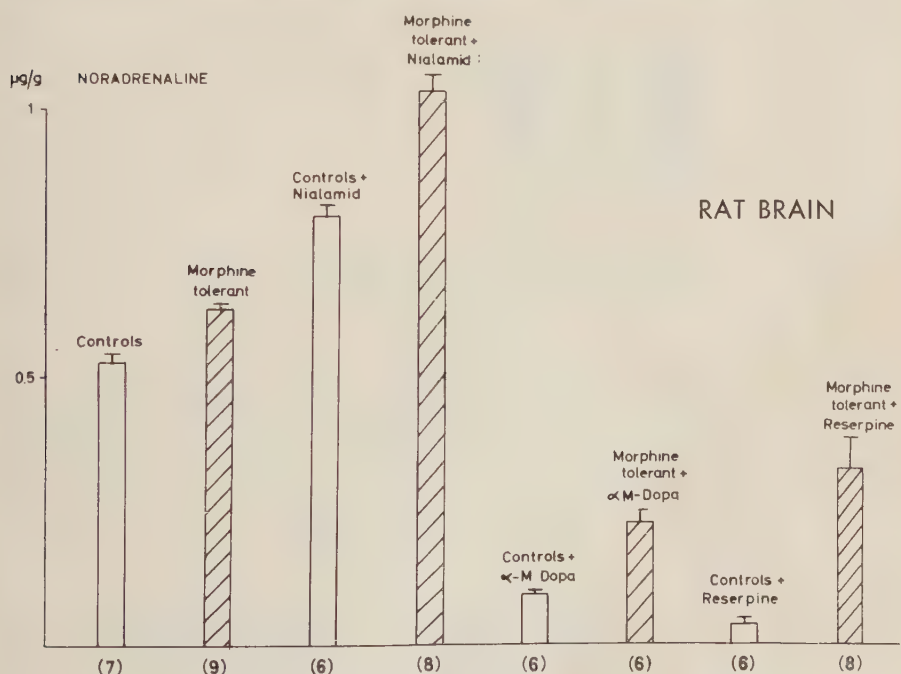


Fig. 7. Effect of saline (white volumns) and chronic morphine treatment (shaded columns) on noradrenaline content of rat brains (means + S.E.M.). Controls and morphine tolerant rats were given: nialamid 300 mg/kg, α -methyl-DOPA 150 mg/kg or reserpine 5 mg/kg, twenty hours before sacrifice. Number of observations within brackets (one observation = two pooled rat brains).

Long-term administration of morphine

No changes in the content of noradrenaline were found in the investigated parts of the *dog* brain as a result of long-term morphine administration as long as the animals were morphine satiated (Table VIII). There were similarly no changes in the content of dopamine in the telencephalon or of 5-hydroxytryptamine in the brain stem (Fig. 8).

In *rats* the brain content of noradrenaline was increased both when the regular dosage regimen was applied (Fig. 7, left) and following the intense dosage regimen (Table IX).

Table X. 5-Hydroxytryptamine (5-HT) content in brain stem of dogs (means $\pm$ S. E. M.). Nalorphine was given 5+5 mg/kg at 2 hours intervals and animals were sacrificed 2 hours after the second injection. Chronic morphine (Mo) treatment was given for 70—90 days, final dose 90—120 mg/kg. Animals sacrificed 20 hours after last injection except in withdrawal group

Nr of dogs	Treatment	5-HT $\mu\text{g/g}$
10	None	0.57 ± 0.034
4	Nalorphine	0.56 ± 0.076
6	Chronic morphine	0.52 ± 0.031
2	Chronic morphine + withdrawal 72 h	0.55
8	Chronic morphine + nalorphine	0.54 ± 0.020

Table XI. 5-Hydroxytryptamine (5-HT) content in whole brains of rats (means $\pm$ S. E. M.). Morphine or saline given twice daily. The animals were sacrificed 4 hours after the last injection, except in the withdrawal group

Nr of rats	Treatment	Final dose mg/kg	5-HT $\mu\text{g/g}$
9	Saline 21 days	—	0.43 ± 0.033
5	Morphine 10 days	60	0.44 ± 0.091
7	Morphine 21 days	180	0.46 ± 0.087
10	Morphine 21 days + Withdrawal 48 h	180	0.42 ± 0.063

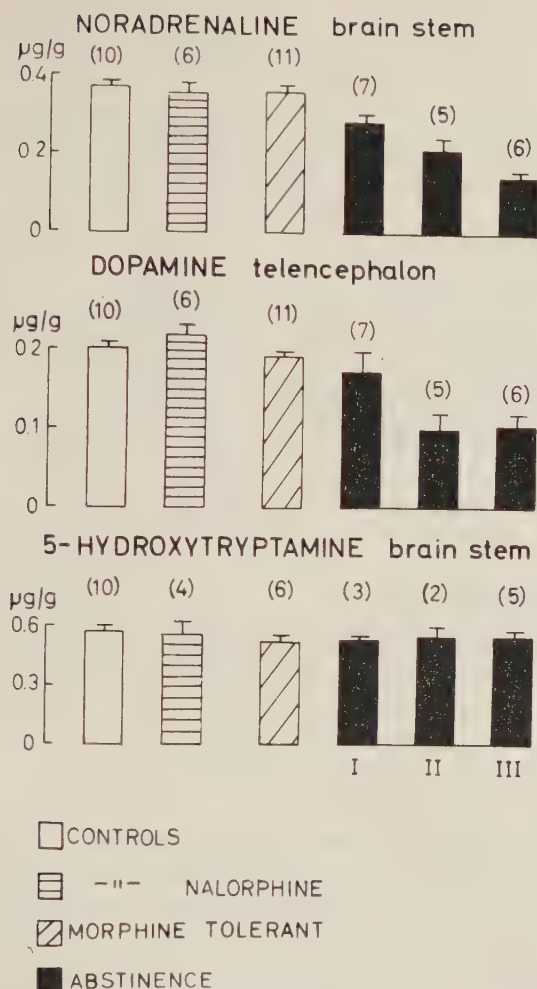


Fig. 8. Effect of morphine tolerance and abstinence, produced by nalorphine or abrupt withdrawal, on the monoamine content in the dog brain (means+S.E.M.). The abstinence group (black columns) was subdivided according to severity of abstinence signs: I slight, II moderate and III marked. Number of animals within brackets.

Fig. 7 illustrates the effect of drug combinations in morphine tolerant rats and controls. The monoamine oxidase inhibitor nialamid produced an elevation of the noradrenaline level in controls, but in morphine tolerant rats there was a significantly larger increase of noradrenaline ($P < 0.001$). Alpha-methyl-DOPA effected a decrease of the noradrenaline level in controls, but in morphine tolerant rats the decrease was smaller ($P < 0.001$).

The same finding was made when the noradrenaline stores were depleted by reserpine. The depletion was significantly smaller in morphine tolerant rats than in controls ($P < 0.001$).

As in dogs, the brain content of 5-hydroxytryptamine in rats was unaltered from chronic morphine administration (Table XI).

Abrupt withdrawal

In two *dogs* the withdrawal from chronic morphine administration for 72 hours resulted in abstinence syndromes of grades II and III. The noradrenaline and dopamine values of these dogs were low (Table VIII), while 5-hydroxytryptamine was unaltered (Table X).

In *rats* the withdrawal for 48 hours did not affect the brain level of noradrenaline (Table IX) or of 5-hydroxytryptamine (Table XI). The abstinence syndrome in the rats was not purely excitatory, but was characterized by a mixture of drowsiness and irritability.

Nalorphine-induced abstinence

In *dogs* given chronic morphine administration the injection of nalorphine gave rise to abstinence phenomena of various intensity, while in controls there were no obvious effects of nalorphine. The brain noradrenaline of nalorphine-injected controls did not differ significantly from untreated controls or morphine tolerant dogs. The abstinence group was subdivided according to the severity of the abstinence phenomena (Grade I—III). In each of these groups there was a significant reduction of the brain stem content of noradrenaline compared with the morphine tolerant group with no signs of abstinence (Fig. 8). The reduction of noradrenaline was 21, 41 and 59 per cent in abstinence of grade I, II and III respectively. The severity of abstinence seemed to be related to the magnitude of the depletion in all parts of the brain. In the telencephalon and cerebellum, however, the relative noradrenaline decrease was even more marked at lower degrees of abstinence (grades I and II) than in the brain stem (Fig. 9).

The dopamine content of the telencephalon was unchanged in controls given nalorphine, but was reduced in connection with abstinence of grade II and III. However, when the abstinence was slight (grade I), there was no significant reduction of the dopamine content (Fig. 8).

The 5-hydroxytryptamine of the brain stem remained unchanged at any degree of abstinence (Fig. 8).

In *rats* there were no changes in the whole brain content of noradrenaline following nalorphine-induced abstinence (Table IX).

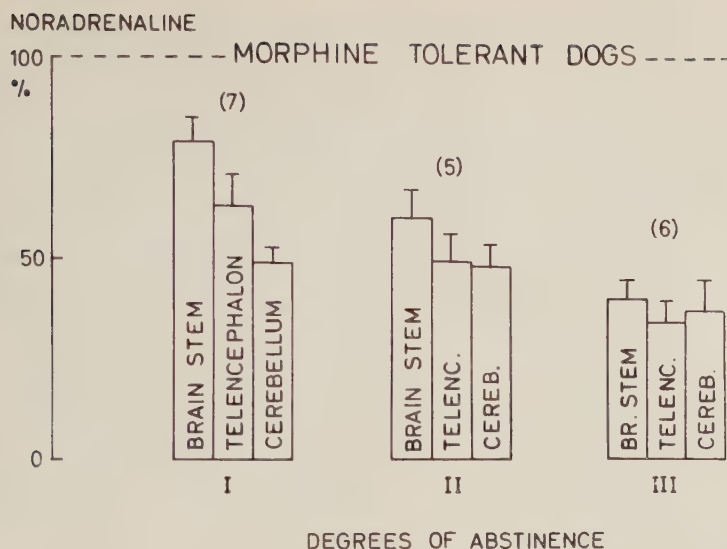


Fig. 9. Noradrenaline in the brain stem, telencephalon and cerebellum of dogs in various degrees of abstinence: I slight, II moderate and III marked abstinence signs. Values expressed as per cent (means + S.E.M.) of the corresponding levels in morphine tolerant dogs without abstinence. Number of animals within brackets.

3. Discussion

The *control levels* of catecholamines and 5-hydroxytryptamine in the brains of dogs, rats and cats were in agreement with those reported from other laboratories (for instance, BERTLER and ROSENGREN 1959, UDENFRIEND 1962, p. 158). The observation of increasing catecholamine concentrations in the developing rat brain (preliminary report in GUNNE 1962 a) is in agreement with the report of KÄRKI, KUNTZMAN and BRODIE (1962).

Seasonal variations in the catecholamines of rat brains were reported by MONTAGU (1956 b) and BEAUVALLET, FUGAZZA and SOLIER (1961). These observations were not confirmed in the present study.

The *acute* administration of morphine has been reported to produce various effects on the brain content of noradrenaline. VOGT (1954) and QUINN *et al.* (1958) obtained a decrease in the cat brain, in agreement with the present results. GUNNE (1959) reported a decrease in rat brains following 30 mg/kg and an increase from higher doses (60—90 mg/kg). FREEDMAN, FRAM and GIARMAN (1961) administered 20 to 60 mg/kg of morphine in rats and found a decrease of noradrenaline after four hours

followed by an increase after 24 to 48 hours. MAYNERT and KLINGMAN (1962) noticed only a decrease of noradrenaline in dog brains from 200 mg/kg and in rat brains from 60—200 mg/kg, while SLOAN *et al.* (1962 b) found an increase in the rat brain after a morphine dose of 60 mg/kg. In monkeys, SEGAL and DENEAU (1962) reported a decrease of brain noradrenaline following 3 mg/kg of morphine, but an increase after 30 mg/kg.

From these rather confusing results it may be concluded that morphine exerts a dual action on the brain content of noradrenaline, some doses producing a rise while others have a tissue depleting effect. Experiments on long-term administration of morphine have supplied evidence that morphine acts by accelerating the synthesis of brain noradrenaline. Such an action from single injections also, might account for the elevated brain levels obtained in some experiments. The depletion of noradrenaline in other experiments indicates a lag of resynthesis behind the utilization, when excessive demands are made by the stimulated tissue. This is generally associated with more or less obvious signs of excitation and is consequently more readily produced in cats than in rats.

A lack of effect of "analgesics" (PLETSCHER, SHORE and BRODIE 1956 a) and of acute morphine administration on the brain content of 5-hydroxytryptamine has been reported in rabbits (BRODIE, SHORE and PLETSCHER 1956), rats and dogs (MAYNERT, KLINGMAN and KAJI 1962). The absence of an effect on 5-hydroxytryptamine together with a depletion of brain catecholamines represents a monoamine response similar to that seen in morphine abstinence in the dog.

After administration of morphine daily for five days the noradrenaline level of the cat's brain stem returned to normal. This indicates a rapid tolerance to the noradrenaline depleting action of morphine.

Following *chronic* morphine administration in dogs the brain content of noradrenaline was normal, in agreement with earlier observations (MAYNERT and KLINGMAN 1962, GUNNE 1962 b). The dog brain content of dopamine and 5-hydroxytryptamine was also unaltered following chronic administration of morphine.

In rats the brain noradrenaline was elevated after 3 weeks of morphine. Approximately the same rise was seen whether morphine was given twice or six times daily.

The species difference observed between dogs and rats with respect to the brain content of noradrenaline in chronic morphine treatment may have some relation to the differences noticed in behavior. Rats were excited from chronic morphine administration while dogs were sedated. Earlier FREEDMAN *et al.* (1961) and MAYNERT and KLINGMAN (1962) reported

a corresponding elevation of brain noradrenaline in rats on tolerance dosage schedules.

Both in dogs and rats, the chronic morphine treatment had produced tolerance to the noradrenaline depletion of the acute experiment. The origin of the supranormal brain levels in morphine tolerant rats was studied further. The mode of action of this tolerance phenomenon could be an interference with 1) the rate of synthesis, 2) the amine binding capacity of the tissue or 3) the rate of metabolic break-down of the brain noradrenaline. Although alternative pathways should be considered, the oxidative deamination by monoamine oxidase is probably the most important route for the metabolic break-down of catecholamines in the brain (p. 11). The monoamine oxidase system seemed to be intact in brains of morphine tolerant rats, since the 5-hydroxytryptamine levels were normal.

When the monoamine oxidase was blocked by a long-acting inhibitor (nialamid), the brain noradrenaline increased more in morphine tolerant than in control rats. MAYNERT and KLINGMAN (1962) obtained the same result with a different monoamine oxidase inhibitor (JB 516). These findings indicate that the difference in brain noradrenaline between controls and morphine tolerant rats is not found in the rate of metabolic break-down.

The possibility that chronic morphine administration had acted on the noradrenaline binding capacity of the brain tissue was tested by administration of two noradrenaline releasing agents, α -methyl-DOPA and reserpine. These compounds are known to reduce the noradrenaline binding capacity of brain tissue, probably by different mechanisms (p. 10). Following administration of either of these drugs, there were still noticed higher noradrenaline levels in morphine tolerant rats than in controls. This indicates that the elevated noradrenaline content in the brains of morphine tolerant rats is probably not explained solely on the basis of an increased storage capacity, but rather by an increased rate of synthesis. On the other hand, an alteration of the catecholamine binding properties in brain tissue is not ruled out (*cf.* p. 75).

In experiments where combinations of drugs have been used, the possibility of a direct interaction and/or competition at receptor sites should also be taken into consideration. That some interaction actually occurs is suggested by the finding of FREEDMAN *et al.* (1961) that morphine could partially reverse the noradrenaline releasing action of reserpine in the brains of rats, whether morphine was given 24 hours before or after reserpine.

In connection with *nalorphine-induced abstinence* there was a reduction

of noradrenaline in the brain stem of dogs. This is in accordance with earlier reports (MAYNERT and KLINGMAN 1962, GUNNE 1962 b).

The present study showed that the severity of the abstinence symptoms was proportional to the depression of noradrenaline. This was true for all parts of the brain, but certain differences seem to exist. The brain stem appeared to be more resistant to the noradrenaline reducing action compared with the cerebellum and telencephalon. Only in cases of marked abstinence were all 3 parts (brain stem, telencephalon and cerebellum) equally depleted. The differences found may reflect different modes of storage. It is known (CARLSSON, FALCK and HILLARP 1962) that the noradrenaline of the brain stem is stored in granules within the nervous tissue. The noradrenaline of cerebellum and telencephalon might be chiefly located at vasomotor nerve endings (*cf.* EULER 1946, VOGT 1954), and these stores could thus be regarded as located peripherally.

The fact that dopamine is also partially depleted in morphine abstinence in the dog can probably not be explained merely on the basis of an increased utilization of the precursor of noradrenaline. The magnitude of the reduction in the forebrain stores of dopamine indicates that this amine is also directly involved in the release. SEGAL and DENEAU (1962) obtained changes in the brain dopamine levels of monkeys at variance with those seen in dogs in the present report. A decrease was noted in morphine dependence, but an increase followed abrupt and nalorphine-induced withdrawal (numerical data were not presented). No explanation for this difference in results can be given at present.

Following *abrupt withdrawal* from chronic morphine administration in dogs, the same general pattern of brain monoamines was noticed as in nalorphine-induced abstinence: a reduction of noradrenaline and dopamine, with unaltered 5-hydroxytryptamine levels.

In rat brains there was no reduction of noradrenaline in withdrawal or nalorphine-induced abstinence, in accordance with MAYNERT and KLINGMAN (1962). In an earlier study (GUNNE 1959) a slight decrease of noradrenaline was noted also in rats following withdrawal. SLOAN *et al.* (1962 a) obtained a reduction of catecholamines in the heart of rats 48 hours after withdrawal from chronic morphine treatment, but found no effect in the brain.

Generally, the effects of morphine abstinence on brain noradrenaline in rats have been small or absent, in contrast with the findings in dogs. The reason for this species difference is not clear, but again it is tempting to correlate the difference with the clinical effects of withdrawal in the two species. Dogs were excited, while rats showed a mixed state of drowsiness

and irritability. If a noradrenaline release actually takes place in the rat brain, however, it might be concealed by the rapid resynthesis in morphine tolerance.

GUNNE (1961) and SLOAN *et al.* (1962 a) reported that morphine withdrawal in rats did not release 5-hydroxytryptamine in the brain. MAYNERT *et al.* (1962) found no effect on brain 5-hydroxytryptamine following nalorphine administration in morphine tolerant dogs and rats. The present investigation confirmed these observations and further showed that 5-hydroxytryptamine was unchanged irrespective of the degree of abstinence intensity.

CHAPTER V

ADRENALINE AND NORADRENALINE IN THE ADRENAL GLANDS IN MORPHINE TOLERANCE AND WITHDRAWAL

The effects of *acute* morphine administration on the content of adrenaline and noradrenaline in the adrenal gland were studied in cats and rats. Dogs and rats were used in studies of the effects of *long-term* morphine administration and *abrupt withdrawal* as well as *nalorphine-induced abstinence*.

1. Special methods

Unilateral sectioning of the splanchnic nerve was performed in a few morphine tolerant dogs. The animals were anaesthetized with pentobarbital (Nembutal, Abbott) 40 mg/kg i. p. and were intubated. Via a midline abdominal incision the left splanchnic nerve was exposed immediately below the diaphragm and was cut. Aseptic conditions were not observed, but 500 000 units of penicillin (Astracillin, Astra Ltd) was given daily for 4 days after the denervation. The animals were sacrificed within 20 days after the denervation, and were then checked for signs of regeneration of the splanchnic nerve. No gross changes in behavior were noted in the operated animals, and no infections occurred.

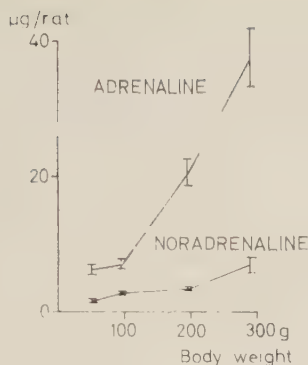
2. Results

Control levels

The adrenaline and noradrenaline content of the adrenal glands, expressed as $\mu\text{g}/\text{pair}$ of adrenals, increased during lifetime in the rats (Fig. 10).

The same tendency was noticed also in adrenal glands of dogs of different age. The material was too small, however, for a statistical treatment and the difference between age-groups was diminished when the catecholamine level was expressed per gram of wet tissue weight (p. 28). In spite of this there was a wide range of the adrenaline content in the adrenals of untreated control dogs (and nalorphine-injected controls): 0.69—2.88 mg/g of wet tissue weight.

Fig. 10. Adrenaline and noradrenaline content of rat adrenals, calculated per pair of adrenals. Means $\pm$ S.E.M. of groups of twelve rats of different body weights. Two pairs pooled for each determination.



Acute and 5 days administration of morphine

Administration of a single morphine dose (30 mg/kg) gave a 70 per cent reduction of adrenaline and an 80 per cent reduction of noradrenaline in the adrenal glands of *cats* sacrificed four hours after the injection. Three animals died from convulsions within 2 to 3 hours after the injection. In two of these cats there was no reduction of the content of adrenaline or

Table XII. Adrenaline (A) and noradrenaline (NA) mg/g tissue weight, in adrenal glands of cats following a single (acute) and 5 days administration of morphine (once daily), 30 mg/kg. Individual values represent means of 2 determinations. (For brains cf Table VI)

Controls		Morphine acute ¹		Morphine acute ²		Morphine for 5 days ²	
A	NA	A	NA	A	NA	A	NA
0.90	0.90	1.64	2.10	0.46	0.12	0.25	0.48
1.04	0.54	1.34	1.12	0.34	0.15	0.16	0.26
1.03	0.70	0.28	0.04	0.36	0.09	0.21	0.23
1.54	0.84			0.13	0.17	0.17	0.60
Means: 1.13 0.75		(1.09) (1.09)		0.32**	0.13***	0.20***	0.39*

¹ Died from convulsions 2—3 hours after injection.

² Sacrificed 4 hours after (last) injection.

* Different from controls $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table XIII. Adrenaline and noradrenaline content in the adrenal glands of rats (means $\pm$ S.E. M.). The rats were given one or two injections of morphine and were sacrificed 4 hours after the first injection. (Second injection was given 2 hours after the first). The adrenals of two rats were pooled for each determination. (For brains cf. Table VII)

Number of rats	Treatment	Dose	Adrenaline $\mu\text{g/rat}$	Noradrenaline $\mu\text{g/rat}$
12	Controls, saline	0.2 ml	26.4 ± 2.0	3.5 ± 0.3
10	Morphine	20 mg/kg	$11.0 \pm 2.7^{**}$	2.6 ± 0.8
10	„	30 mg/kg	$12.4 \pm 1.8^{***}$	2.8 ± 0.6
10	„	30 + 30 mg/kg	$3.9 \pm 1.5^{***}$	$1.4 \pm 0.7^*$

* Different from controls $P < 0.05$.

** „ „ „ $P < 0.01$.

*** „ „ „ $P < 0.001$.

noradrenaline (Tab. XII). When the same morphine dose had been given daily for five days the mean adrenaline level was lower than in the acute experiment while the noradrenaline level showed a beginning return (different from acute experiment: $P < 0.05$).

When morphine was given to *rats* in acute experiments there was a reduction of adrenaline with doses of 20 to 60 mg/kg, and a dose—response relationship was established. (The last-mentioned dose was divided in 30 + 30 mg/kg). The noradrenaline content was reduced only following the highest dose (Table XIII).

Long-term administration of morphine

No changes were found in the adrenaline and noradrenaline content of the adrenal glands in *dogs* (Fig. 11) or *rats* (Table XV) rendered morphine tolerant by long-term administration.

Abrupt withdrawal

In *dogs* an abrupt withdrawal, resulting in abstinence symptoms of grade II and grade III, was accompanied by a reduced adrenaline content (two animals), Table XIV.

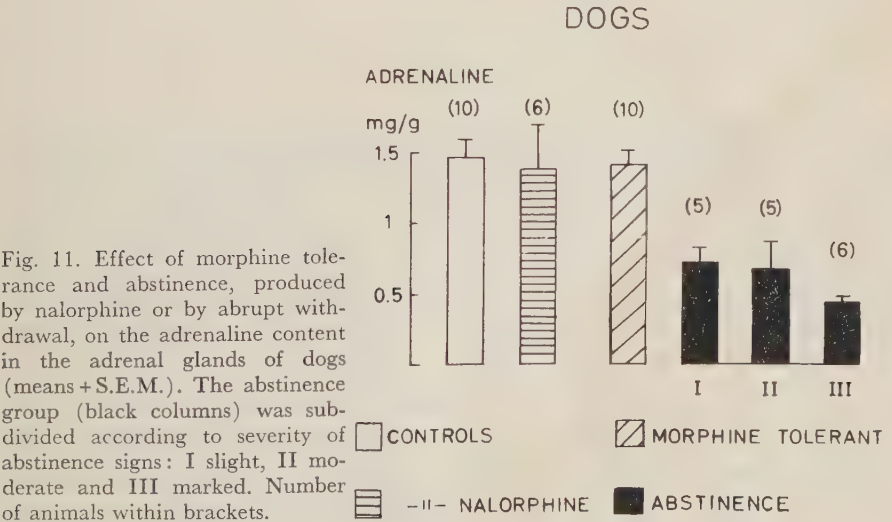
In *rats* withdrawal also caused a marked decrease in adrenaline content. Forty-eight hours after abrupt withdrawal the mean adrenaline content was 48 per cent of that found in adrenals of morphine tolerant rats (Table XV). The abstinence symptoms of these rats, treated by the intense regimen

Table XIV. Adrenaline and noradrenaline content and weight of adrenal glands of dogs (means $\pm$ S.E. M.). Nalorphine was given 5+5 mg/kg at 2 hours intervals and animals were sacrificed 2 hours after the second injection. Chronic morphine (Mo) treatment was given for 70—90 days final dose 90—120 mg/kg. Animals sacrificed 20 hours after last morphine injection, except in the withdrawal group

Number of dogs	Treatment	Weight g	Adrenaline mg/g	Noradrenaline mg/g
10	None	1.12 $\pm$ 0.11	1.37 $\pm$ 0.11	0.23 $\pm$ 0.03
6	Nalorphine	1.24 $\pm$ 0.11	1.39 $\pm$ 0.31	0.25 $\pm$ 0.03
10	Chronic Mo	1.15 $\pm$ 0.13	1.41 $\pm$ 0.10	0.25 $\pm$ 0.03
2	Chronic Mo+ withdrawal 72 h	1.30	0.22	0.09
14	Chronic Mo+ nalorphine	1.36 $\pm$ 0.11	0.60 $\pm$ 0.08***	0.15 $\pm$ 0.02

*** Different from chronic Mo: P < 0.001.

(p. 14), was a combination of drowsiness and irritability. The noradrenaline content of the adrenals was mainly unaffected in both species.



Nalorphine-induced abstinence

In morphine tolerant dogs the injection of nalorphine induced abstinence syndromes of various degrees and a marked decrease of adrenaline in the adrenal glands. The noradrenaline content was not significantly decreased. In non-tolerant dogs there were no behavioral effects of nalorphine and the adrenaline and noradrenaline content was normal. (Table XIV.) The

Table XV. Adrenaline (A) and noradrenaline (NA) content in the adrenal glands of rats (means $\pm$ S. E. M.). Chronic morphine treatment was given for 3 weeks, up to a final dose of 300 mg/kg, given 6 times daily at 4 hours intervals (1 800 mg/kg day). The rats were killed 4 hours after the last injection of saline or nalorphine with exception of the withdrawal group. Adrenals of 2 rats pooled for each determination (cf. Table IX for brains of same rats)

Number of rats	Treatment	A μ g/rat	NA μ g/rat
10	Saline	24.5 $\pm$ 1.33	4.4 $\pm$ 0.68
10	Nalorphine 10 mg/kg	22.0 $\pm$ 1.58	5.7 $\pm$ 0.45
12	Chronic morphine + saline 1 inj.	26.8 $\pm$ 1.73	4.2 $\pm$ 0.77
12	Chronic morphine + nalorphine 10 mg/kg	23.1 $\pm$ 1.86	3.7 $\pm$ 0.52
6	Chronic morphine + withdrawal 48 h	13.9 $\pm$ 1.93**	2.2 $\pm$ 0.52

** Different from chronic morphine: $P < 0.01$.

abstinence group was subdivided according to the degree of abstinence and Fig. 11 shows that the largest reduction of adrenaline content occurred in animals which exhibited the most pronounced abstinence symptoms. The reduction of adrenaline was 49, 52 and 68 per cent in abstinence of grades I, II and III respectively.

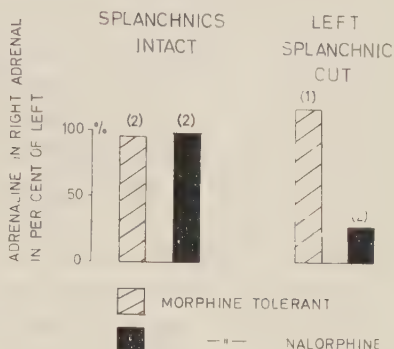
In rats the adrenaline and noradrenaline content was not correspondingly reduced by nalorphine (Table XV). No clearcut excitatory abstinence was seen following nalorphine in morphine tolerant rats.

Unilateral splanchnic section

Results of experiments on three morphine tolerant dogs in which the left splanchnic nerve had been cut are shown in Fig. 12. One of these dogs

was given saline and two received nalorphine 5 + 5 mg/kg, with resulting abstinence signs (right in Fig. 12). For comparison four non-operated dogs were included, two morphine tolerant and two morphine tolerant + nalorphine (left in Fig. 12). The figure demonstrates that the adrenaline con-

Fig. 12. Adrenaline content of the right adrenal, expressed in per cent of adrenaline content of left adrenal gland of dogs. *Note that after denervation of the left adrenal there is a marked reduction of the adrenaline content only in the innervated (right) adrenal after nalorphine-induced abstinence.* Numbers of dogs within brackets.



tent was almost equal on the right and left side in all groups, the only exception being the abstinence group in which the nerve had been cut.

In these dogs the adrenaline level was definitely subnormal on the right, innervated side (0.16 and 0.18 mg/g), in agreement with the findings reported above for morphine tolerant dogs given nalorphine. In the denervated gland probably no reduction of adrenaline had occurred. The values were essentially normal, although in the lower part of the normal range (0.66 and 0.84 mg/g).

3. Discussion

The *control* values for adrenaline and noradrenaline in adrenal glands of untreated dogs, rats and cats are in fair agreement with earlier reports (*cf.* EULER 1956, p. 113—114). HÖKFELT (1951) has given an account of the catecholamine content of rats from foetal to adult stage. The present report confirms the finding of a rapid increase, especially of adrenaline, in the developing rat adrenal. This finding emphasized the importance of using control rats, preferably of approximately the same age in every long-term morphine experiment.

The *acute* administration of morphine to cats and rats reduced the adrenal gland content of adrenaline and noradrenaline. This effect appeared after a latent period, since two cats which died from convulsions 2 to 3 hours after the morphine administration had normal values for both

amines. After four hours, however, all values were depressed. VOGT (1954) reported a corresponding lag in the depletion of brain and adrenal catecholamines after administration of various drugs, including morphine, in cats. The exact meaning of this lag time is obscure. It would appear from this study that the depletion is a rapid process which takes place 3 to 4 hours after the morphine injection.

After daily administration of morphine to cats for five days a beginning replenishment of adrenal noradrenaline was observed. This is in accordance with the data of BUTTERWORTH and MANN (1957 a, b), and BERTLER, HILLARP and ROSENGREN (1960), who found that stimulation of the adrenal medulla resulted in an increased synthesis of noradrenaline. Any stimulation producing an increased output of adrenaline would be likely to cause a rise of the resynthesis of noradrenaline in the adrenal glands. This may explain the findings in dogs and rats that when adrenaline was reduced, the noradrenaline level generally remained normal. In acute experiments on rats only the intense stimulation of two consecutive morphine injections also produced a decline of noradrenaline.

MAYNERT and KLINGMAN (1962), in acute morphine experiments obtained a decrease in the adrenal gland content of adrenaline in dogs and rats only after higher morphine doses. (200 mg/kg in dogs, 60—200 mg/kg in rats). The present study demonstrates that there are probably strain differences between rats with respect to the acute effects of morphine, since the rats of this study responded already, in the same way, to doses of 20 and 30 mg/kg of morphine.

In *morphine tolerant* dogs and rats the catecholamine content was normal. This finding shows that tolerance had developed to the adrenaline reducing effect of the acute experiments.

When abstinence was induced by *abrupt withdrawal*, there was a marked reduction of the adrenaline content of the adrenals both in dogs and rats. *Nalorphine-induced abstinence* reduced the adrenaline content only in dogs in agreement with MAYNERT and KLINGMAN (1962) and GUNNE (1962 b). If the abstinence symptoms of the dogs were graded according to severity (grades I—III), it was seen that the catecholamine reduction was largest in the group with the most pronounced symptoms (grade III). Nalorphine was without effect in the adrenals of morphine tolerant rats. This need not, however, be interpreted as a principal difference between dogs and rats with regard to the catecholamine response during abstinence. It was noted that a depletion of adrenaline actually occurred also in rats, when the chronic morphine treatment was abruptly withdrawn, indicating that an adrenaline release was involved in both species.

The mechanism of action of the adrenaline depletion in nalorphine-induced abstinence in the dog was elucidated by the experiment, in which one adrenal gland had been denervated. Normally the right and left adrenals contain equal amounts of adrenaline, as shown in cats (BUTTERWORTH and MANN 1957 c) and dogs (GUNNE, to be publ.). This was found to be true in non-operated dogs, both in morphine tolerance and when the adrenaline content had been depleted by abstinence. Unilateral denervation did not seem to influence the adrenal gland content of adrenaline in the morphine tolerant dog without abstinence signs. When nalorphine was given, however, a depletion was produced on the innervated side. The sectioning of the splanchnic nerve on one side had probably prevented the adrenaline release in the denervated gland during nalorphine-induced abstinence.

This finding suggests that the depletion of adrenaline in the adrenal medulla seen in morphine abstinence is produced indirectly through the secretory nerves.

CHAPTER VI

URINARY EXCRETION OF ADRENALINE, NOR- ADRENALINE, AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN MORPHINE TOLERANCE AND WITHDRAWAL

The urinary excretion of adrenaline, noradrenaline and 5-HIAA was studied in dogs and rats. When morphine was administered daily in constant and later stepwise increasing doses, information was obtained concerning the secretion during *acute* as well as *chronic administration*. Finally the secretion during and following *abrupt withdrawal* and *nalorphine-induced abstinence* were studied.

1. Results

Resting levels of catecholamines and 5-HIAA

After an acclimation period of one week in the metabolic cages, stable control values were obtained in four young *dogs* (litter mates), two males and two females weighing 3—5 kg (Table XVI). The urinary output of adrenaline and noradrenaline in the dogs was approximately the same in males and females. On the other hand, during six control days the excretion of 5-HIAA was consistently higher in the two females ($9.0 \pm 0.7 \mu\text{g/kg/h}^1$), than in the two males ($3.5 \pm 0.3 \mu\text{g/kg/h}^1$). Despite these individual differences, the values of all four dogs were calculated together.

Four groups of *rats* were employed, weighing 150—170 g at the beginning of the experiment (Table XVI). Three groups were later given chronic morphine treatment, while one group was kept as control throughout the experiment. The control levels of catecholamines were derived from all four groups before morphine treatment and from the control group during the whole experiment.

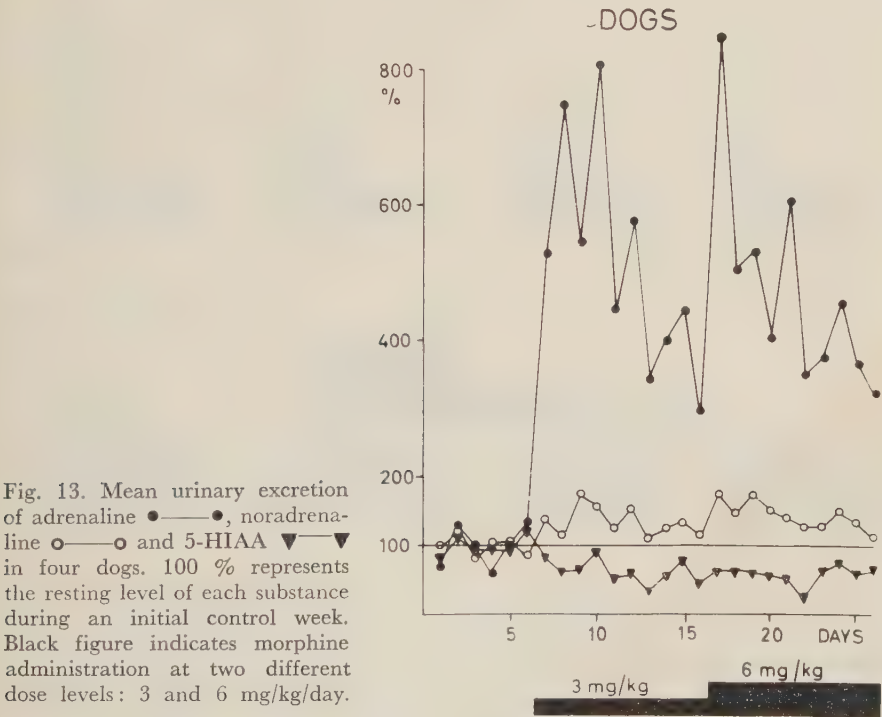
Initial morphine dose

In *dogs* the injection once daily of 3 mg/kg of morphine gave a five to eight-fold increase of the adrenaline output (absolute values: 41—63 $\mu\text{g/}$

¹ Mean $\pm$ S. E. M.

Table XVI. Urinary control levels of adrenaline (A), noradrenaline (NA) and 5-hydroxyindoleacetic acid (5-HIAA) in dogs and rats

Species (number of animals)	Number of observa- tions	Excretion product	Urinary output Mean $\pm$ S.E.M.	(S.D.)
Dog (4)	24	A	7.8 $\pm$ 0.8	(4.0) ng/kg/h
	24	NA	31.6 $\pm$ 1.9	(9.3) ng/kg/h
	24	5-HIAA	6.2 $\pm$ 0.7	(3.3) μ g/kg/h
Rat (20)	35	A	4.9 $\pm$ 0.22	(1.3) ng/rat/h
	35	NA	22 $\pm$ 0.80	(4.8) ng/rat/h
	9	5-HIAA	1.50 $\pm$ 0.079	(0.24) μ g/rat/h



kg/h), lasting for four days. After this the values tended to return and on the tenth day the rise was only three-fold (Fig. 13). The noradrenaline output followed essentially the same pattern, but the percentage changes

were small compared with adrenaline (absolute values: 36—56 $\mu\text{g/kg/h}$). The urinary 5-HIAA was diminished during morphine administration.

In *rats* the same pattern of excretion was obtained for adrenaline and noradrenaline. The first two daily injections of morphine, 20 mg/kg, gave a rise of both catecholamines, but one week later the same dose had a much smaller effect (Fig. 14).

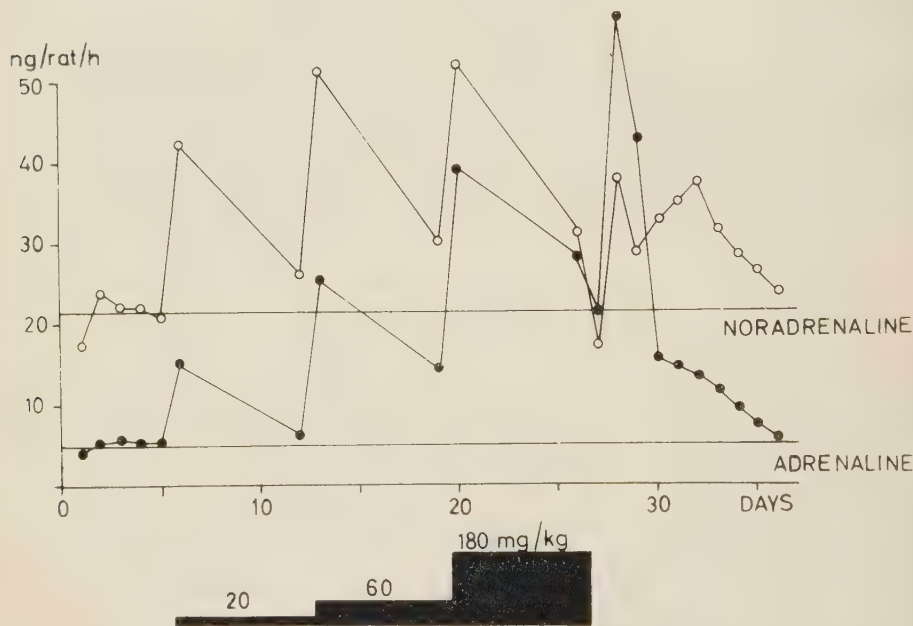


Fig. 14. Urinary adrenaline ●—● and noradrenaline ○—○ excretion in rats before, during and after a three weeks cycle of morphine. Each value represents mean of three groups of rats. Control levels indicated by horizontal lines. Black figure: morphine doses given twice daily.

Stepwise increase of morphine dose

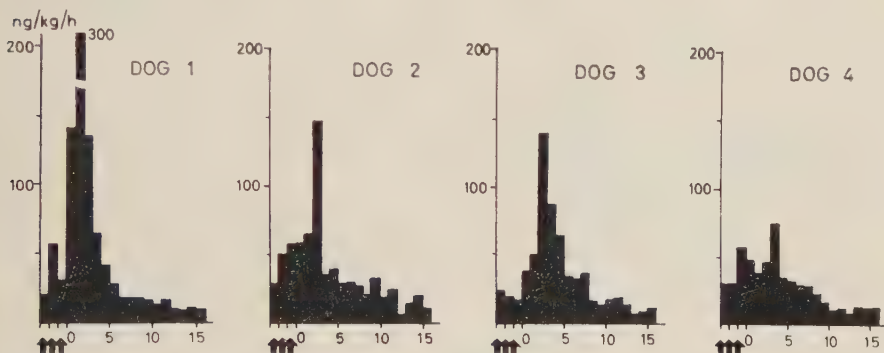
Whenever the morphine dose was raised, stepwise, the same pattern of an initial increase followed by another decrease reappeared in *dogs* (Fig. 13) and *rats* (Fig. 14). There was not, however, a complete normalization of the adrenaline output as long as morphine was given, while the noradrenaline level returned within the normal range both in *dogs* and *rats*.

The 5-HIAA excretion remained low in *dogs*, but was found to be elevated in *rats* following chronic morphine administration (see below).

Abrupt withdrawal

When the chronic morphine administration was abruptly withdrawn there was a rise in the urinary output of adrenaline and noradrenaline in *dogs*. All four dogs showed only slight abstinence symptoms (grade I) and in no case was there any decrease of body weight or rise in body temperature (recorded daily by a thermocouple inserted 5 cm into the rectum). No major differences in symptoms could be seen between the four dogs during withdrawal, but the adrenaline rise showed rather great individual variations (75–300 ng/kg/h) (Fig. 15). The noradrenaline rise was more

ADRENALINE



NORADRENALINE

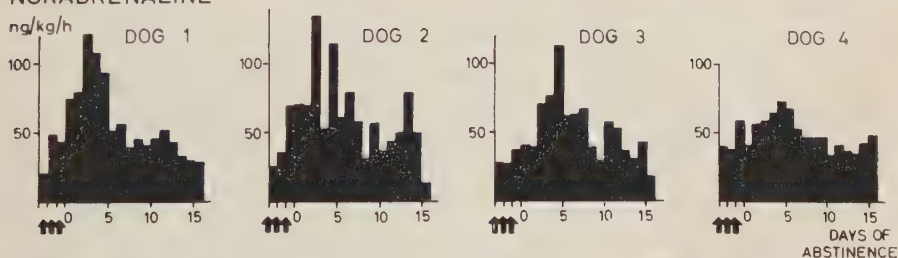
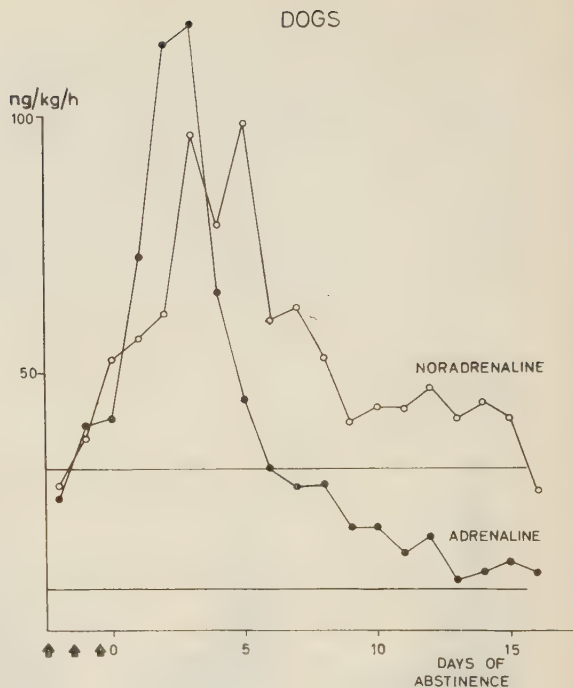


Fig. 15. Urinary excretion of adrenaline (upper figures) and noradrenaline (lower figures) of individual dogs during withdrawal from chronic morphine treatment. Arrows indicate the last three injections of morphine 90 mg/kg.

evenly distributed between the dogs (73–135 ng/kg/h). The mean excretion curves (Fig. 16) reached a maximum for adrenaline on the third day of withdrawal, followed by a rapid decline. The noradrenaline excretion reached a first maximum on the third day of withdrawal, simultaneously with the adrenaline maximum. On the same day the abstinence symptoms were most prominent.

Fig. 16. Mean urinary excretion of adrenaline ●—● and noradrenaline ○—○ in four dogs during withdrawal from chronic morphine treatment. Arrows indicate the last three daily injections of morphine 90 mg/kg. Horizontal lines indicate mean control levels of the same dogs before morphine administration.



On the fifth day, when the adrenaline was rapidly decreasing, there was a second rise in the noradrenaline excretion. The output of adrenaline and noradrenaline returned to normal after 13 to 15 days of withdrawal.

The output of 5-HIAA rose above the control level on the first day of withdrawal but on the following days it was normal (Fig. 17).

In *rats* the urinary adrenaline and noradrenaline followed essentially the same pattern as described in dogs (Fig. 14). The adrenaline rise in rats was maximal on the second day of withdrawal, when the symptoms were most pronounced. At the same time there was a first maximum of noradrenaline output.

A few days later, when the adrenaline excretion was showing a rapid decline, there was a second rise of noradrenaline, this time without further abstinence symptoms. The output of adrenaline and noradrenaline returned to normal after 10 days of withdrawal.

The excretion of 5-HIAA, which was high on the last three days of chronic morphine administration, became subnormal on the first two days of withdrawal. It remained normal during the rest of the withdrawal period (Fig. 18).

Fig. 17. Mean urinary excretion of 5-HIAA in four dogs during the last three days of a cycle of addiction and ten days of withdrawal. Each arrow indicates injection of morphine 90 mg/kg.

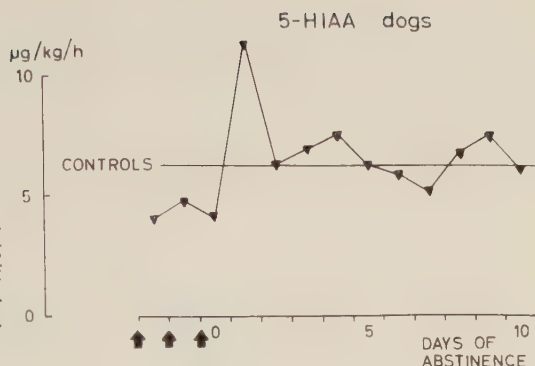
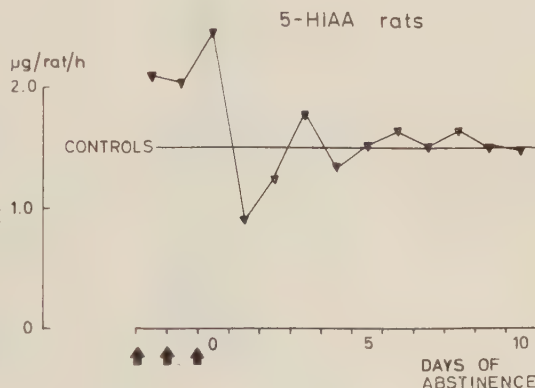


Fig. 18. Mean urinary excretion of 5-HIAA in three groups of (five) rats during the last three days of a cycle of addiction and ten days of withdrawal. Each arrow indicates injection of morphine 180 mg/kg.



Nalorphine-induced abstinence

In dogs the effect of nalorphine (10 mg/kg) was initially studied in non-tolerant animals during the control period. Nalorphine was without effect on the output of noradrenaline and 5-HIAA in non-tolerant dogs, but gave a rise of adrenaline (to 37** ng/kg/h) on the day of injection. An injection of saline was without effect. No symptoms were noticed following nalorphine in these dogs before morphine treatment.

After chronic morphine administration, nalorphine (10 mg/kg) produced a moderate abstinence syndrome (grade II) in all four dogs. Fig. 19 (left) illustrates the effect on the daily output of adrenaline, noradrenaline and 5-HIAA. When one daily morphine injection (90 mg/kg) was substituted by nalorphine, there was a rise of all three excretion products. Adrenaline increased nine-fold on the day of nalorphine, from 29 ng/kg/h (during morphine treatment) to 260 ng/kg/h (= 25 µg/dog/24 h). When the interrupted morphine administration was continued, the adrenaline rise dis-

** Different from controls $P < 0.01$.

appeared again. The noradrenaline rise was five-fold, from 27 ng/kg/h (during morphine treatment) to 130 ng/kg/h (= 12 μ g/dog/24 h). When the morphine injections were reinstituted it was found that the noradrenaline rise outlasted the adrenaline rise by two days. The output of 5-HIAA was doubled on the day of nalorphine.

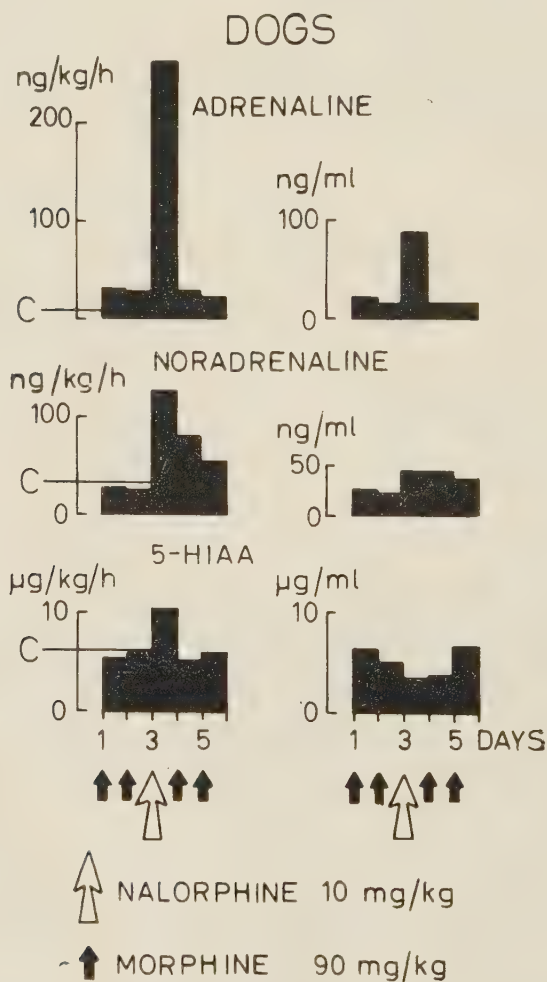


Fig. 19. Left figures: mean urinary excretion of adrenaline, noradrenaline and 5-HIAA in four dogs made tolerant to 90 mg/kg of morphine daily by injections for two months. C=control levels of same animals before morphine treatment. On the third day of experiment the daily morphine injections were substituted by nalorphine 10 mg/kg. Right figures: urinary concentration of each substance during the same experiment.

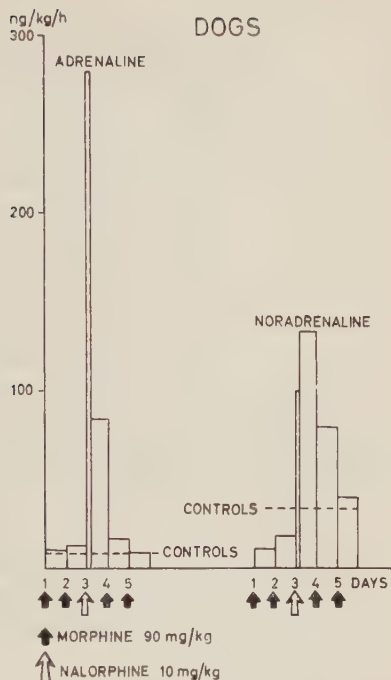


Fig. 20. Urinary excretion of adrenaline (left) and noradrenaline (right) in morphine tolerant dog. On the third day of the experiment the daily morphine injections were substituted by 10 mg/kg nalorphine. The first four hours portion of urine following nalorphine was assayed separately.

The concentration (per ml of urine) of adrenaline and noradrenaline rose as a result of nalorphine (Fig. 19, right), while there was a decrease of the 5-HIAA concentration. The changes in diuresis are discussed in the following section.

An attempt was made to determine the sequence of events with regard to catecholamine excretion in this nalorphine experiment. The urine of the first four hours following the injection of nalorphine was analyzed separately and the output of the next twenty hours in a second portion. In two of the animals there was a conspicuous rise of adrenaline already in the first portion (exemplified in Fig. 20). The noradrenaline rise in all animals was highest during the following twenty hours.

(Following nalorphine in morphine-tolerant *rats* there was diarrhea and a great reduction of the urine volumes. This made a satisfactory collection of urine impossible in such experiments on rats).

2. Diuresis

The effects of morphine on the urine volumes in *dogs* are illustrated in Fig. 21. The daily morphine injections diminished the output of urine

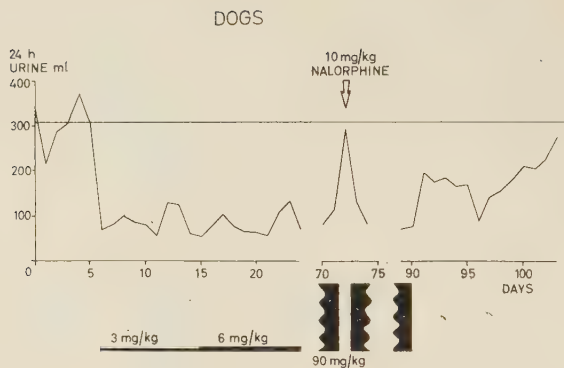


Fig. 21. Daily urine volumes ml/dog/24 hours (means of four dogs) before and during chronic morphine administration (black figures: daily morphine doses). Effect of nalorphine and abrupt withdrawal when the dogs had been made tolerant to 90 mg/kg of morphine. Horizontal line: mean control level before treatment.

to about one third of the control level. No obvious signs of tolerance to this effect were noticed during the ten days when the initial morphine dose was maintained.

Nalorphine temporarily inversed the antidiuretic effect of morphine, thus producing a rise of the urine volumes compared with the adjacent days of morphine treatment. After withdrawal there was another rise on the first day, but the urine volumes were still subnormal. Following another few days of low output there was a return towards the control level.

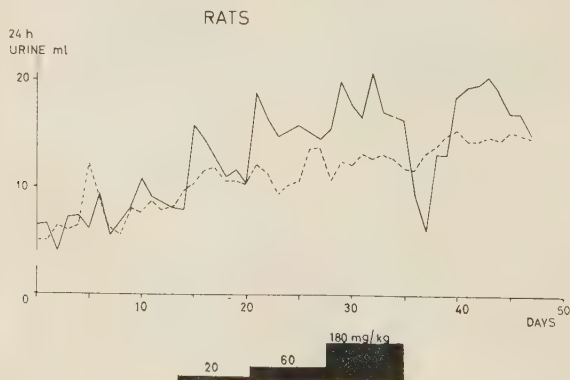


Fig. 22. Daily urine volumes ml/rat/24 hours, before, during and after a three weeks cycle of morphine. Broken line: saline treated controls (one group of five rats). Solid line: morphine treated (means of three groups of rats). Black figure: morphine doses, given twice daily.

In *rats* the urine volumes were increased during chronic morphine administration, as compared with saline-injected controls.

After withdrawal there were diminished urine volumes in the rats on the first two days of abstinence, followed by another rise (Fig. 22). As the rats were gaining weight during the experiments, from 160 to 250 g, there was a gradual increase of urine volume also in the controls.

3. Discussion

Resting levels of adrenaline and noradrenaline in the urine of rats showed a good agreement with earlier reports in which a similar technique was employed (SCHAPIRO 1958, PERMAN 1961, LEDUC 1961). The catecholamine values in dogs were considerably lower (about 1/10) than those reported by LEROY and SCHAEPPDRYVER (1961). These authors used adult dogs, while the present study was made on puppies.

Earlier reports containing control values for urinary 5-HIAA in non-anaesthetized dogs (ERSPAMER and CICERI 1957, RENSON 1960) and rats (ERSPAMER 1954 b, BERTACCINI and ERSPAMER 1962), were in agreement with those of the present paper.

The two male dogs excreted consistently less than half the amount of 5-HIAA compared with the two females. UDENFRIEND, TITUS and WEISSBACH (1955) have pointed out that there are great individual variations in the excretion of 5-HIAA in dogs. Whether there is an actual sex difference, as has been reported for histamine excretion in rats (NETTER, COHN and SHORE 1961), remains an open question.

In non-tolerant dogs and rats the *acute* injection of morphine resulted in an increased output of adrenaline and noradrenaline. When the same dose had been repeated for some days, the excretion of both catecholamines were showing a return towards normal levels. The urinary adrenaline remained supranormal as long as morphine was given, while noradrenaline decreased to normal levels after *long-term* administration of the drug. In both species the same cyclic pattern of excretion, a rise followed by another decrease, was seen whenever the morphine dose was increased stepwise.

The experiments indicate that the catecholamine production and/or release are involved in the mechanisms concerned with adaptation of the organism to morphine. Whether there is a decline of the initial release when the morphine injections are repeated, or an increased enzymatic destruction of the free amines, remains to be settled.

After *withdrawal* from chronic morphine administration there was a

greatly increased output of adrenaline and noradrenaline in dogs and rats. The maximal symptoms of abstinence in both species coincided with the highest adrenaline excretion and a simultaneously appearing first outburst of noradrenaline excretion. The magnitude of the adrenaline secretion was apparently unrelated to the abstinence symptoms of the four dogs. It may be assumed that the differences in adrenaline output reflects merely temporary differences in the size of the stores of the adrenal glands. A wide range was found for adrenaline in the adrenal glands of dogs. — A few days later in the withdrawal period there was a second rise of noradrenaline both in dogs and rats. By that time the adrenaline excretion was rapidly declining and the symptoms of abstinence were fading.

The first rise of adrenaline excretion, appearing simultaneously with several obvious signs of increased autonomic activity, is probably to be ascribed to a release at adrenergic nerve terminals throughout the organism. The second increase of noradrenaline may have a different explanation.

As already argued in the previous chapter (p. 58) the intense stimulation of the adreno-medullary system, effecting a depletion of the adrenaline content of the adrenal glands, elicits an increased synthesis of its precursor noradrenaline. If the adrenal gland is further stimulated after depletion, it would be likely to discharge more noradrenaline than otherwise. Thus, the second increase of urinary noradrenaline during withdrawal from morphine, may be derived from the partly exhausted adrenal glands.

Before the morphine administration in non-tolerant dogs, a single injection of *nalorphine* only gave a small rise of urinary adrenaline (37 ng/kg/h), while noradrenaline and 5-HIAA were not affected. In morphine tolerant dogs the output of adrenaline rose to a mean of 260 ng/kg/h (controls 7.8 ng/kg/h) and noradrenaline to 130 ng/kg/h (controls 32 ng/kg/h) on the day of nalorphine injection. These high rates of output correspond to a mean of 25 μ g adrenaline and 12 μ g noradrenaline per dog in one day. Assuming a urinary excretion of 2 to 3 per cent of the adrenaline released from the adrenal glands into the blood stream (EULER 1956, p. 285), the adrenaline output following nalorphine would correspond to 0.8—1.2 mg adrenaline released in each dog during 24 hours. The adrenals of these dogs were later found to contain on the average 1.1 mg/dog. A release of this magnitude would be likely to exhaust the adrenaline content of the adrenal glands unless the resynthesis were to be very rapid.

The “immediate” response to nalorphine (first four hours after injection) in some morphine tolerant dogs was dominated by adrenaline while the maximal noradrenaline increase appeared later. Analogously with the findings in withdrawal experiments, the noradrenaline rise outlasted the adrena-

line rise by 1 to 2 days. The nalorphine-induced abstinence symptoms, on the other hand, lasted only two hours after the injection. This indicates that part of the noradrenaline may originate from the adrenal glands, depleted of their adrenaline stores.

In all experiments on urinary excretion products in morphine administration and withdrawal, fluctuations in the urine volumes may imply sources of error. The findings of EULER (1956, p. 167), PERMAN (1961) and LEDUC (1961) indicate that the daily urinary excretion of catecholamines is largely uninfluenced by physiological variations of the diuresis. Great artificial increases in the urinary flow may, however, bring about an increased output of catecholamines. LEROY and SCHAEPPDRYVER (1961), on the other hand, reported a close parallelism between the urine volumes and the output of catecholamines in dogs.

In the present study it was noticed that increases in the urine volumes in dogs and rats during the control period, were generally accompanied by reduced catecholamine concentrations. This suggests that the daily excretion of amines was largely uninfluenced by changes in diuresis. Such a view was further supported by a comparison between dogs and rats during chronic morphine treatment and withdrawal. The patterns of catecholamine excretion were mainly identical, although the fluctuations in diuresis were moving in opposite directions in the two species.

Recently it has been shown that urinary 5-HIAA can be greatly increased in rats given water by stomach tube and that the excretion of this acid is largely dependent on the diuresis both in rats (BERTACCINI and ERSPAMER 1962) and dogs (LEWIS 1959).

The changes in urine volumes observed in dogs and rats during administration of morphine and during withdrawal are in accordance with earlier reports (the matter is reviewed by SCHAUHMANN 1957, p. 70). The increased intake of water and the increased urine volumes of morphine tolerant rats (BARBOUR *et al.* 1929) may be connected with the fever produced by the drug in this species (GUNNE 1960).

The output of 5-HIAA was decreased in dogs and increased in rats during chronic morphine administration. On the first 1 to 2 days of withdrawal, these excretion patterns were reversed: an increase was seen in dogs and a decrease in rats. The concomitant changes in urine volumes support the view that the fluctuations in 5-HIAA were secondary to changes in the diuresis.

The nalorphine-induced abstinence in the dog was accompanied by a rise of both catecholamines and 5-HIAA, together with an increased diuresis. Despite the large urine volumes, the concentration of noradrenaline and

adrenaline was increased per ml of urine, indicating that the increased output of these amines could not be explained solely as an effect of diuresis. 5-HIAA, on the other hand, was decreased when calculated per ml of urine. The rise in 5-HIAA might thus have been secondary to the nalorphine-induced rise in diuresis.

CHAPTER VII

GENERAL DISCUSSION

The results presented have shown that biologically active monoamines in the brain form characteristic patterns of distribution and release both during long-term treatment with morphine and after withdrawal. Marked alterations have been found in the catecholamine content of brain, adrenals and urine, whereas the 5-hydroxytryptamine metabolism remained essentially unaffected.

A comparison was performed between two methods for the estimation of catecholamines in brain extracts, *viz.* the method of BERTLER *et al.* (1958) and the method of EULER and LISHAJKO (1961). It was found that the extraction fluid (0.4 N perchloric acid) recommended by BERTLER *et al.* (1958) for catecholamines and by BERTLER (1961) for 5-hydroxytryptamine was not a suitable medium for the long-term storage of the amines. A slow gradual reduction of noradrenaline was noticed, when extracts were stored for more than a week at -30° C. The disappearance of 5-hydroxytryptamine occurred at a much higher rate and was noticeable already a few hours after extraction. If, however, the pH of the extract was adjusted to 3.5—4.0 the catecholamines were stable for several months and 5-hydroxytryptamine for more than three weeks. In the method of EULER and LISHAJKO (1961) the addition of the disodium salt of ethylenediaminetetraacetic acid (EDTA) to the brain extracts was omitted, since it was found to reduce the noradrenaline values considerably. When these and a few other minor methodological modifications were observed, both methods gave essentially the same results with regard to accuracy and specificity for the purpose of the present investigation.

The *acute* administration of morphine was associated with a decrease of catecholamines in brain and adrenals (observed in rats and cats), together with an increased urinary output of catecholamine (observed in dogs and rats). These findings confirm earlier reports of a catecholamine releasing action of morphine in acute experiments (*cf.* p. 11). In rats the injection of 20 or 30 mg/kg of morphine gave a reduction of the adrenal gland content of adrenaline. The brain stores of noradrenaline, on the other

hand, were unaffected by these doses and were reduced only after two consecutive injections of 30 + 30 mg/kg. MAYNERT and KLINGMAN (1962) have recently reported a correspondingly dissociated catecholamine response in brain and adrenals after acute morphine administration in dogs and rabbits, small doses giving a depression of adrenal but not of brain catecholamines. In rats they noticed only a lack of correlation between the magnitude of change in the two organs. These observations indicate either a greater discharge from the adrenals, or a slower rate of resynthesis compared with the brain.

After administration of morphine and other sympathetic stimulating agents in cats VOGT (1954) found "a correlation between the power of a drug to cause loss of noradrenaline from the hypothalamus and loss of amines from the innervated adrenal medulla". The present experiments confirmed these observations in cats, since the acute administration of 30 mg/kg of morphine produced a 50—80 % reduction of catecholamines both in brain and adrenals. After five days of morphine treatment a difference between the relative catecholamine content in brain and adrenals became evident also in this species. A replenishment had occurred in the brain noradrenaline, while the adrenal glands were still depleted of adrenaline. However, as evidence of a beginning replenishment of catecholamines in the adrenal glands as well, there was a slight increase of noradrenaline, compared with the acute experiment.

The differences noticed between brain and adrenals in acute (rats) and 5 days experiments (cats), thus may be explained by different turn-over rates of catecholamines in the two organs.

There have been differences in opinion concerning the rate of resynthesis of catecholamines both in the brain and in the adrenal glands. Some observers (BUTTERWORTH and MANN 1957 a, b, EADE and WOOD 1958) found the turn-over rate of the adrenals to be slow, while others have concluded that it is rapid (*cf.* BYGDEMAN, EULER and HÖKFELT 1960). The brain was reported to have a rapid turn-over of catecholamines (CARLSSON, LINDQVIST and MAGNUSSON 1959, SPECTOR, MELMON and SJOERDSMA 1962), while BRODIE, SPECTOR and SHORE (1959 b) found it to be relatively slow. BERTLER (1961) concluded that the turn-over rate of the rabbit brain is faster than that of the adrenals. Whether differences in turn-over rates in brain and adrenals represent the only explanation of the present results, remains an open question.

In *long-term* experiments on dogs and rats the catecholamines in brain, adrenals and urine tended to return to control levels.

In rats the noradrenaline depletion of the acute experiment was even converted into an increase of the brain levels. The 5-hydroxytryptamine level remained unchanged, however, indicating that the monoamine oxidase system is intact. It was shown that the raised level of noradrenaline was still present in morphine tolerant rats (compared with controls) after administration of drugs interfering with the storage capacity (α -methyl-DOPA and reserpine) and metabolic break-down (the monoamine oxidase inhibitor nialamid). These findings seem to suggest that the main difference between controls and morphine tolerant rats is not found in the rate of metabolic break-down. Thus, one probable explanation of the elevated brain level of noradrenaline in morphine tolerant rats may be an increased rate of synthesis.

On the other hand the possibility of an increased storage capacity should not be disregarded for the following reasons. It was found that nialamid produced a state of alertness and excitation in rats after a lag period of a few hours. This stage of increased alertness is generally ascribed to an overflow of free catecholamines in the brain tissue (BRODIE *et al.* 1959 b). Since the lag time was prolonged in morphine tolerant rats compared with controls, this would indicate an increased catecholamine binding capacity rather than an increased rate of synthesis in the brains of morphine tolerant animals. Possibly both mechanisms are at work at the same time.

In morphine tolerant dogs there was no corresponding increase in the brain content of catecholamines. This finding may be a correlate to the behavioral differences between the two species. Dogs are sedated, while rats are excited from long-term morphine administration.

The adrenal gland content of adrenaline was normalized during chronic morphine treatment both in dogs and rats in the present study. MAYNERT and KLINGMAN (1962), however, reported an increase of the adrenal gland content of catecholamines in rats and rabbits during chronic morphine treatment. No explanation for this difference in results can be given at present.

The urinary catecholamines, which were elevated by the first morphine injections, showed a decline as a result of tolerance. The noradrenaline output gradually became normal and adrenaline was stabilized on a slightly elevated rate of excretion which seemed to be maintained as long as morphine was given. These findings suggest that the adaptation to long-term morphine administration is associated with a reduced liberation of catecholamines compared with the acute experiments. The return to normal of the brain and adrenal tissue levels during chronic morphine administration thus may be explained, not only on the basis of a stimulated

resynthesis, but partly as the result of a decreased release of catecholamines.

The brain level of 5-hydroxytryptamine in dogs and rats was unaffected by chronic morphine administration. Minor fluctuations in the urinary output of 5-hydroxyindoleacetic acid (5-HIAA) might well be explained by changes in the diuresis. The findings seemed to indicate that 5-hydroxytryptamine was not involved in the monoamine release, nor were there any signs of an accelerated production of this substance in chronic morphine administration. This is in agreement with earlier findings in acute and long-term morphine experiments (p. 12).

Abrupt withdrawal and *nalorphine-induced abstinence* were associated with a rapid mobilization of the catecholamine stores in brain and adrenals. There was a reduction of noradrenaline and dopamine in the brain and of adrenaline in the adrenal glands of dogs, together with an increased urinary output of adrenaline and noradrenaline. The reduction of adrenaline in the adrenal gland was probably prevented by sectioning of the ipsilateral splanchnic nerve (2 dogs) and could thus be regarded as a central effect, mediated through nervous stimulation.

The close relationship between a release of catecholamines in the brain and certain states of excitation with autonomic disturbance, has been demonstrated in many earlier investigations (p. 9). In those earlier studies the role of the catecholamines in the observed syndrome was sometimes obscure, owing to the presence of various drugs which may have exerted a direct action on the nervous tissue. In the present investigation a catecholamine release was noticed in two different experimental situations: 1) In acute experiments a release was seen in the non-tolerant organism in the *presence* of morphine and 2) following abrupt withdrawal a corresponding release was seen in morphine tolerant animals in the *absence* of the drug. The symptom pictures were different in these two instances. In the acute experiment there was depression succeeded by excitation in the rats, but only excitation in cats. In non-tolerant dogs given a large dose (200 mg/kg) of morphine MAYNERT and KLINGMAN (1962) noticed "mild convulsions", together with a decrease of catecholamines in brain and adrenals. Thus, the symptoms of the acute experiments vary, but generally include some signs of excitation. In the withdrawal experiments on dogs the symptoms are purely excitatory and the catecholamine pattern in brain, adrenals and urine indicates a rapid release. These findings support the view that a central liberation of catecholamines is associated with signs of excitation, but that the presence of a drug can obscure the symptom picture.

The close connection between the catecholamine release in morphine withdrawal and the symptoms of excitation appears from 1) the agreement between the *degree* of abstinence intensity and the degree of reduction of the catecholamines in the brain, and 2) the coincidence in *time* of the maximal intensity of the abstinence symptoms and the maximal urinary output of catecholamines.

In morphine tolerant rats and dogs the urinary output of adrenaline and noradrenaline was maximal on the second and third days of abrupt withdrawal, and at this time the adrenaline content of the adrenal glands was reduced. In dogs the most prominent excitatory symptoms in withdrawal and nalorphine-induced abstinence were connected with the most pronounced depletion in the brain catecholamines (noradrenaline and dopamine). In rats there was no depletion of brain noradrenaline in the present abstinence experiments. The symptoms consisted of a combination of drowsiness and irritability together with several autonomic manifestations. In earlier experiments a small reduction of brain noradrenaline was noticed in a different rat strain during withdrawal from chronic morphine administration (GUNNE 1959). A moderate noradrenaline release, in the brain of morphine tolerant rats, need not deplete the brain stores, since the release might well be concealed by the rapid resynthesis of brain noradrenaline. As mentioned previously the long-term morphine experiments in rats (including a final injection of nialamid, α -methyl-DOPA or reserpine) have given evidence of an increased rate of synthesis of noradrenaline in the brain.

Before definite conclusion can be drawn from changes in the tissue levels of monoamines, some information must be obtained concerning the rate of metabolism of the substances studied. Such information is furnished by studies of the urinary output, which readily reflects changes in the rate of synthesis and liberation (*cf.* EULER 1956, p. 285). The urinary content of catecholamines reflects those changes more "dramatically" than the total metabolic end-products, but of course they give no information concerning the catabolic routes. It is true that the monoamines of the brain do not appear in the urine (they do not readily pass the blood-brain barrier; furthermore they are quantitatively insignificant). However, second-hand information may be obtained also concerning the neurochemical events in the brain, since a stimulation of hypothalamic brain centers which liberates the brain catecholamines is generally reflected by a concomitant liberation from adrenals and peripheral adrenergic nerve terminals (VOGT 1959).

MAYNERT and KLINGMAN (1962) reported distinct species differences in the catecholamine response of dogs and rats, based on studies of tissue

levels. They concluded that the adaptations on withdrawal of morphine "are not stressful in the rat, but are in the dog . . ." However, the present investigation has shown, mainly by studies of the urinary output, that there are probably no principal species differences between dogs and rats with regard to the catecholamines in morphine abstinence. On the contrary a conspicuous *similarity* was found between the patterns of catecholamine output in the two species during withdrawal from chronic morphine treatment.

5-Hydroxytryptamine seemed to be unaltered also in connection with morphine abstinence. The brain levels were unchanged during abstinence irrespective of the intensity of symptoms. Changes in the urinary 5-HIAA were probably secondary to the great fluctuations in diuresis connected with withdrawal or nalorphine-induced abstinence.

The exact role of the catecholamine release in the production of the morphine withdrawal syndrome is difficult to decide at present. Many stressful agents cause an increased activity in the sympathetic nervous system and increase the secretion from the adrenal glands. It should be recalled, however, that the drug-induced depletion of hypothalamic noradrenaline, first reported by VOGT (1954) was not elicited by all the drugs tested, in spite of the use of sublethal doses. Only when more or less conspicuous symptoms of autonomic activity were evoked, a depletion of brain noradrenaline was found. Thus, the drugs active on hypothalamic catecholamines had the common feature of stimulating sympathetic activity. VOGT (1957) showed that nalorphine counteracted the behavioral as well as the noradrenaline depleting effects of morphine in the cat brain. Again the effect on catecholamines was associated with the autonomic and behavioral response. MAYNERT and KLINGMAN (1962) were able to prevent the noradrenaline depletion of nalorphine in the brain of morphine tolerant dogs, when pentobarbital anaesthesia was given, in order to abolish the abstinence excitation.

Together with the present results these findings suggest that the release of brain catecholamines in morphine abstinence is secondary to a vigorous stimulation of sympathetic brain centers. However, the concept of a nervously mediated stimulation as the cause of the catecholamine depletion, does not contradict the view that a liberation of these substances in the brain stem, might have a decisive influence on the symptom pattern in morphine abstinence.

The possible role of other biologically active substances in the brain must be clarified before the relation between brain catecholamines and morphine effects can be finally evaluated. MAYNERT *et al.* (1962) reported that the

brain content of γ -aminobutyric acid (GABA) was unchanged in morphine abstinence in the dog. — It has been shown that the stress of morphine withdrawal involves an increased activity also of the adrenal cortex both in man (EISENMAN *et al.* 1958, EISENMAN, FRASER and BROOKS 1961) and in rats (PAROLI and MELCHIORRI 1961). In view of the clinical effects of administered corticosteroids it seems improbable, however, that these hormones could explain the symptom pattern in morphine abstinence.

HIMMELSBACH (1943) remarked that the symptoms of morphine abstinence are indicative of a "disturbance in the function of the vegetative nervous system", the homeostasis of which was connected with "autonomic hypothalamic centers". — Recently the catecholamine and possibly also the 5-hydroxytryptamine stores of the brain stem nuclei were visualized by histochemical fluorescence technique (FALCK 1962, CARLSSON, FALCK and HILLARP 1962). They concluded that "adrenergic neurons may exist and noradrenaline may serve as a synaptic transmitter in the central nervous system". Their observations provide direct evidence for a transmitter function of the central monoamines and thus strengthen the hypothesis that the specific alterations in brain noradrenaline content during morphine administration and morphine abstinence reflect biochemical neuronal changes in the regions which control autonomic activity. It also emerges from the present investigation that, in spite of species differences, there are several common features in the neurochemical patterns associated with morphine administration and abstinence in dogs and rats and that minor differences may be correlated to behavioral differences. This implies a probability that the present results will facilitate an understanding of the corresponding mechanisms in human morphine addicts.

SUMMARY

1. The effects of long-term administration of morphine and of abrupt withdrawal as well as of nalorphine-induced abstinence have been studied, mainly with reference to the brain content of noradrenaline, dopamine and 5-hydroxytryptamine (serotonin), the adrenal gland content of adrenaline and noradrenaline and the urinary output of adrenaline, noradrenaline and 5-hydroxyindoleacetic acid (5-HIAA). The study was carried out on dogs, rats and cats. The brain noradrenaline was determined either according to BERTLER, CARLSSON and ROSENGREN (1958) or EULER and LISHAJKO (1961). These methods were compared and a few modifications were suggested.

2. The general appearance and behavior of the animals were noticed during acute and long-term administration of morphine and in abstinence produced by nalorphine or by abrupt withdrawal. The abstinence syndrome in rats was found to contain some apparently inconsistent elements: signs of excitation (irritability and disturbed autonomic functions), together with drowsiness and eyelid ptosis. The abstinence syndrome in dogs, however, was purely excitatory. A simple rating scale for abstinence symptoms in dogs was devised. The symptoms were scored as slight (grade I), moderate (grade II) or marked (grade III).

3. *Brain:* *Acute* administration of morphine reduced the brain noradrenaline in rats and cats. After five daily morphine injections the brain noradrenaline was restored in cats. Following *chronic* morphine treatment the brain level of noradrenaline, dopamine and 5-hydroxytryptamine was normal in dogs. In rats the noradrenaline level was supranormal after chronic morphine treatment, while the brain content of 5-hydroxytryptamine was unaltered. A monoamine oxidase inhibitor (nialamid) caused a greater increase of noradrenaline in morphine tolerant rats than in controls. The noradrenaline releasing agents α -methyl-DOPA and reserpine produced a smaller noradrenaline decrease in brains of morphine tolerant rats than in controls.

During *withdrawal* and *nalorphine-induced abstinence* there was in dogs a reduction of noradrenaline in all parts of the brain (telencephalon, brain

stem and cerebellum). The magnitude of the reduction was related to the severity of the abstinence symptoms. In the brain stem there was a 21, 41 and 59 per cent reduction in abstinence in grades I, II and III respectively. A concomitant reduction of dopamine was seen in dogs with moderate and marked (grade II and III) abstinence symptoms, but did not appear when the symptoms were slight (grade I). The 5-hydroxytryptamine of the brain stem was unaltered at any degree of abstinence. — In rats there were no changes of brain noradrenaline or 5-hydroxytryptamine during morphine abstinence.

4. *Adrenals*: *Acute* administration of morphine reduced the adrenaline and noradrenaline content of the adrenal glands in rats and cats. After five days of morphine administration in cats there was still a reduction of both amines, but a beginning replenishment of noradrenaline was noticed. After *chronic* morphine administration in dogs and rats a normal content of adrenaline and noradrenaline was found in the adrenal glands.

Following *withdrawal* of chronic morphine administration there was a reduction of adrenaline in the adrenal glands of dogs and rats. *Nalorphine-induced abstinence* reduced the adrenaline level in the adrenals of dogs. There was a 49, 52 and 68 per cent reduction in abstinence in grades I, II and III respectively. The adrenaline depletion was probably mediated *via* the splanchnic nerves, since after unilateral splanchnic sectioning only the innervated gland contained definitely subnormal amounts of adrenaline (two dogs). — In morphine tolerant rats nalorphine did not influence the catecholamine content of the adrenals.

5. *Urine*: The *first* few morphine injections caused an increase of the urinary output of adrenaline and noradrenaline in dogs and rats. After *repeated* injections there was a return of the two catecholamines towards the normal level. The noradrenaline output returned to normal, while adrenaline remained supranormal in both species as long as morphine was given. The output of 5-HIAA during long-term morphine administration appeared only to reflect changes in the diuresis both in dogs and rats.

Following *abrupt withdrawal* there was a marked rise of urinary adrenaline and noradrenaline in dogs and rats. The increase of noradrenaline outlasted the increase of adrenaline by 2 to 3 days in both species. A corresponding pattern of catecholamine excretion was also noticed in dogs when abstinence was produced by *nalorphine*. — The urinary 5-HIAA rose in morphine tolerant dogs on the first day of withdrawal or administration of nalorphine, but a decrease was seen in rats. Again the fluctuations of urinary 5-HIAA might have been secondary to changes in diuresis, since

corresponding alterations were noticed in the urine volumes of the two species.

6. The following *general conclusions* are drawn from the present findings:

a) Acute administration of morphine produces an activation of sympathetic parts of the nervous system, central as well as peripheral, eventually leading to a depletion of brain and adrenal stores of catecholamines in rats and cats. Long-term administration of morphine induces an increased rate of resynthesis of catecholamines, probably as a response to the increased demands by the stimulated tissue. Signs of an accelerated resynthesis are found in brain and adrenals of rats and cats and in the adrenals of dogs during chronic morphine treatment.

b) Withdrawal and nalorphine-induced abstinence elicits a rapid liberation of brain and adrenal catecholamines and the *intensity* of the abstinence syndrome in dogs is proportional to the depletion of brain catecholamines. Furthermore the maximal symptoms coincide in *time* with the maximal urinary output of catecholamines. There are species differences between dogs and rats with regard to the behavioral manifestations of abstinence. These are reflected by quantitative differences in the catecholamine depletion between the two species. The general patterns of catecholamine output following morphine withdrawal are, however, essentially the same in dogs and rats.

These findings seem to link the appearance and development of the morphine abstinence syndrome with a liberation of brain catecholamines (noradrenaline and dopamine). 5-Hydroxytryptamine appears to remain unaffected by chronic morphine treatment as well as by withdrawal.

ACKNOWLEDGEMENTS

The present investigation was carried out in the years 1958 to 1962 during the tenure of a research fellowship from Karolinska Institutet.

I wish to express my sincere gratitude to Professor U. S. v. Euler, head of the Department of Physiology, for his invaluable help and advice during these years.

To Professor L. Goldberg, head of the Department of Alcohol Research, my thanks are due for valuable and stimulating discussions and for the financial support generously given me by his institution.

I also wish to thank Mr John Jonsson for his invaluable and skilful technical assistance.

The determinations of 5-HIAA were carried out by Bengt Pernow M.D., to whom I wish to express my appreciation.

The investigation was supported chiefly by a research grant from the Alcohol Research Committee of the Swedish Medical Research Council. I have also received research grants from Svenska Sällskapet för Medicinsk Forskning, Stiftelsen Lars Hiertas Minne and Stiftelsen Bror Gadeliuss Minnesfond. All these grants are gratefully acknowledged.

I also wish to thank Pfizer, Ltd., for supplying Niamid, Ciba, Ltd. for Serpasil and Merck, Sharp & Dohme, Ltd. for Aldomet.

REFERENCES

- ABE, T., Veränderungen des Adrenaliningehaltes in der Nebenniere bei akuter und chronischer Morphinvergiftung. *Abstr. Jap. J. Med. Sci.* 1929. 4, 100—104.
- AMIN, A. H., T. B. B. CRAWFORD and J. H. GADDUM, The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol. (Lond.)* 1954. 126. 596—618.
- AXELROD, J. A., Possible mechanism of tolerance to narcotic drugs. *Science* 1956. 124. 263—264.
- AXELROD, J. A., Presence, formation and metabolism of normetanephrine in the brain. *Science* 1958. 127. 754—755.
- AXELROD, J. A., Metabolism of epinephrine and other sympathomimetic amines. *Physiol. Rev.* 1959. 39. 751.
- AXELROD, J. A., G. HERTTING and R. W. PATRICK, Inhibition of H^3 -norepinephrine release by monoamine oxidase inhibitors. *J. Pharmacol. exp. Ther.* 1961. 134. 325—328.
- BARBOUR, H. G., B. E. RUSSEL, S. H. FLOWERS, E. S. DUNHAM and L. G. HUNTER, The significant redistribution of water between internal and surface tissues and the blood at the height of morphine withdrawal, *Amer. J. Physiol.* 1929.90. 273—274.
- BARLOW, O. W., The tranquilizing potency of morphine, pantopon, codeine, papaverine and narcotine. *J. Amer. Med. Ass.* 1932. 99. 986—988.
- BEAUVALLET, M., J. FUGAZZA and M. SOLIER, Variations saisonnières du taux de la noradrénaline cérébrale chez le rat. *C. R. Soc. Biol. (Paris)*. 1961. 155. 1465—1466.
- BERTACCINI, G. and V. ERSPAMER, The effect of the administration of water or isotonic NaCl solution on the urinary excretion of 5-hydroxyindoleacetic acid in the rat. *J. Pharm. and Pharmacol.* 1962. 14. 687—697.
- BERTLER, Å., A. CARLSSON and E. ROSENGREN, Method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.* 1958. 44. 273—292.
- BERTLER, Å. and E. ROSENGREN, Occurrence and distribution of catecholamines in brain. *Acta physiol. scand.* 1959. 47. 350—361.
- BERTLER, Å., *Inverkan av reserpin och andra läkemedel på katekolaminomsättningen*. Diss. Lund. 1960.
- BERTLER, Å., N.-Å. HILLARP and E. ROSENGREN, "Bound" and "free" catecholamines in the brain. *Acta physiol. scand.* 1960. 50. 113—118.
- BERTLER, Å., Effect of reserpine on the storage of catecholamines in brain and other tissues. *Acta physiol. scand.* 1961. 51. 75—83.
- BLASCHKO, H., The specific action of 1-dopa decarboxylase. *J. Physiol (Lond.)* 1939. 96. 50P—51P.
- BRODIE, B. B., A. PLETSCHER and P. A. SHORE, Evidence that serotonin has a role in brain function. *Science* 1955. 122. 968.
- BRODIE, B. B., P. A. SHORE and A. PLETSCHER, Serotonin-releasing activity limited to Rauwolfia alkaloids with tranquilizing action. *Science* 1956. 123. 992—993.
- BRODIE, B.B., E. G. TOMICH, R. KUNTZMAN and P. A. SHORE, On the mechanism of action of reserpine: effect of reserpine on capacity of tissues to bind serotonin. *J. Pharmacol. exp. Ther.* 1957. 119. 461—467.
- BRODIE, B. B., S. SPECTOR and P. A. SHORE, Interaction of drugs with norepinephrine in the brain. *Pharmacol. Rev.* 1959 a. 11. 548—564.
- BRODIE, B. B., S. SPECTOR and P. A. SHORE, Interaction of monoamine oxidase inhibitors with physiological and biochemical mechanisms in brain. *Ann. New York Acad. Sci.* 1959 b. 80. 609—614.

- BRODIE, B. B., R. KUNTZMAN, C. W. HIRSCH and E. COSTA, Effects of decarboxylase inhibition on the biosynthesis of brain monoamines. *Life Sciences*. 1962. 1. 81—84.
- BUTTERWORTH, K. R. and M. MANN, The adrenaline and noradrenaline content of the adrenal gland of the cat following depletion by acetylcholine. *Brit. J. Pharmacol*. 1957 a. 12. 415—421.
- BUTTERWORTH, K. R. and M. MANN, The release of adrenaline and noradrenaline from the adrenal gland of the cat by acetylcholine. *Brit. J. Pharmacol*. 1957 b. 12. 422—426.
- BUTTERWORTH, K. R. and M. MANN, A quantitative comparison of the sympathomimetic amine content of the left and right adrenal glands of the cat. *J. Physiol. (Lond.)* 1957 c. 136. 294—299.
- BYGDEMAN, S., U. S. v. EULER and B. HÖKFELT, Resynthesis of adrenaline in the rabbits adrenal medulla during insulin-induced hypoglycemia. *Acta physiol. scand*. 1960. 49. 21—28.
- CARLSSON, A., M. LINDQVIST and T. MAGNUSSON, 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature. (Lond.)* 1957. 180. 1 200.
- CARLSSON, A., M. LINDQVIST, T. MAGNUSSON and B. WALDECK, On the presence of 3-hydroxytyramine in brain. *Science* 1958. 127. 471.
- CARLSSON, A. and B. WALDECK, A fluorimetric method for the determination of dopamine (3-hydroxytyramine). *Acta physiol. scand*. 1958. 44. 293—298.
- CARLSSON, A., M. LINDQVIST and T. MAGNUSSON, The effect of monoamine oxidase inhibitors on the metabolism of the brain catecholamines. *J. Soc. Ciencias Med. Lisboa* 1959. 123. Suppl. 96.
- CARLSSON, A. and M. LINDQVIST, In-vivo decarboxylation of α -methyl DOPA and α -methyl metatyrosine. *Acta physiol. scand*. 1962. 54. 87—94.
- CARLSSON, A. and N.-Å. HILLARP, Formation of phenolic acids in brain after administration of 3, 4-dihydroxyphenylalanine. *Acta physiol. scand*. 1962. 55. 95—100.
- CARLSSON, A., B. FALCK and N.-Å. HILLARP, Cellular localization of brain monoamines. *Acta physiol. scand*. 1962. 56. Suppl. 196.
- COCHIN, J. and J. AXELROD, Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine and normorphine. *J. Pharmacol. exp. Ther.* 1959. 125: 2. 105—110.
- COHEN, G. and M. GOLDENBERG, The simultaneous fluorimetric determination of adrenaline and noradrenaline in plasma. — I. *J Neurochem*. 1957. 2. 58—70.
- COSTA, E., G. L. GESSA, C. HIRSCH, R. KUNTZMAN and. B. B. BRODIE, On current status of serotonin as a brain neurohormone and in action of reserpinelike drugs. *Ann. N. Y. Acad. Sci.* 1962. 96. 118—133.
- CRAWFORD, T. B. B. and W. LAW, The urinary excretion of adrenaline and noradrenaline by rats under various experimental conditions. *Brit. J. Pharmacol*. 1958. 13. 35—43.
- DEGWITZ, R., R. FROWEIN, C. KULENKAMPFF and U. MOHS, Über die Wirkungen des L-DOPA beim Menschen und deren Beeinflussung durch Reserpin, Chlorpromazin, Iproniazid und Vitamin B₆. *Klin. Wschr.* 1960. 38. 120—123.
- DETRICK, L. E. and C. H. THIENES, Tissue hydration during morphine addiction and withdrawal in rats on low calcium diet and on high calcium diet with parathyroid hormone injections. *Arch. int. Pharmacodyn.* 1941. 66. 130—137.
- EADE, N. R. and D. R. WOOD, The release of adrenaline and noradrenaline from the adrenal medulla of the cat during splanchnic stimulation. *Brit. J. Pharmacol*. 1958. 13. 390—394.
- EHRLEN, I., Fluorimetric determination of adrenaline II. *Farm. Rev. (Stockh.)* 1948. 47. 242—250.
- EISENMAN, A., H. FRASER, J. SLOAN and H. ISBELL, Urinary 17-ketosteroid excretion during a cycle of addiction to morphine. *J. Pharmacol, exp. Ther.* 1958. 124. 305—311.

- EISENMAN, A., H. FRASER and J. BROOKS, Urinary excretion and plasma levels of 17-hydroxycorticosteroids during a cycle of addiction to morphine. *J. Pharmacol. exp. Ther.* 1961. 132. 226—231.
- ELLIOTT, T. R., The action of adrenaline. *J. Physiol. (Lond.)*. 1905. 32. 401—467.
- ELLIOTT, T. R., The control of the suprarenal glands by the splanchnic nerves. *J. Physiol. (Lond.)* 1912. 44. 374—409.
- EMMELIN, N. and R. STRÖMBLAD, Adrenaline and noradrenaline content of the suprarenals of cats in chloralose and morphine-ether anaesthesia. *Acta physiol. scand.* 1951. 24. 260—266.
- ERSPAMER, V., Pharmacology of indolealkylamines. *Pharmacol. Rev.* 1954 a. 6. 425—487.
- ERSPAMER, V., Observations on the metabolism of endogenous 5-hydroxytryptamine (enteramine) in the rat. *Experientia (Basel)*. 1954 b. 10. 471—472.
- ERSPAMER, V. and C. CICERI, Action of reserpine on the 5-hydroxytryptamine (enteramine) biosynthesis and metabolism in dogs and rats. *Experientia* 1957. 13. 87—89.
- EULER, U. S. v., A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relations to adrenaline and noradrenaline. *Acta physiol. scand.* 1946. 12. 73—97.
- EULER, U. S. v. and I. FLODING, A fluorimetric micromethod for differential estimation of adrenaline and noradrenaline. *Acta physiol. scand.* 1955. 33. Supp. 118: 45—56.
- EULER, U. S. v., *Noradrenaline*, Thomas, Springfield, Ill. 1956.
- EULER, U. S. v., Distribution and metabolism of catechol hormones in tissues and axones. *Rec. Progr. Horm. Res.* 1958. 14. 483—512.
- EULER, U. S. v. and F. LISHAJKO, The estimation of catechol amines in urine. *Acta physiol. scand.* 1959. 45. 122—132.
- EULER, U. S. v. and F. LISHAJKO, Improved technique for the fluorimetric estimation of catecholamines. *Acta physiol. scand.* 1961. 51. 348—355.
- FALCK, B., Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta physiol. scand.* 1962. 56. Suppl. 197.
- FELDBERG, W. and C. C. TOH, Distribution of 5-hydroxytryptamine (serotonin, enteramine) in the wall of the digestive tract. *J. Physiol. (Lond.)* 1953. 119. 352—362.
- FISHER, R. A., *Statistical methods for research workers*. Edinburgh 1936.
- FREEDMAN, D. X., D. H. FRAM and N. J. GIARMAN, The effect of morphine on the regeneration of brain norepinephrine after reserpine. *Fed. Proc.* 1961. 20. 321.
- GADDUM, J. H., Drugs antagonistic to 5-hydroxytryptamine. *Ciba Foundation Symposium, Hypertension*. London. 1954. p. 75—77.
- GESSA, G. L., E. COSTA, R. KUNTZMAN and B. B. BRODIE, Evidence that loss of brain catecholamine stores due to blockade of storage does not cause sedation. *Life Sciences*. 1962. 1. 441—452.
- GEY, K. F. and A. PLETSCHER, Activity of monoamine oxidase in relation to the 5-hydroxytryptamine and norepinephrine content of the rat brain. *J. Neurochem.* 1961. 6. 239—243.
- GUNNE, L.-M., Noradrenaline and adrenaline in the rat brain during acute and chronic morphine administration and during withdrawal. *Nature (Lond.)* 1959. 184. 1950—1951.
- GUNNE, L.-M., The temperature response in rats during acute and chronic morphine administration. A study of morphine tolerance. *Arch. int. Pharmacodyn.* 1960. 129. 416—428.
- GUNNE, L.-M., The excretion of noradrenaline and adrenaline in the urine of rats during chronic morphine administration and during abstinence. *Psychopharmacologia (Berl.)* 1961. 2. 214—220.
- GUNNE, L.-M., Catecholamine metabolism and morphine abstinence. *Ann. N.Y. Acad. Sci.* 1962 a. 96. 205—210.

- GUNNE, L.-M., Catecholamine metabolism in morphine withdrawal in the dog. *Nature (Lond.)* 1962 b. 195. 815—816.
- GUNNE, L.-M., Relative adrenaline content in brain tissue. *Acta physiol. scand.* 1962 c. 56. 324—333.
- HÄGGENDAL, J., On the use of strong exchange resins for determinations of small amounts of catecholamines. *Scand. J. Clin. Lab. Invest.* 1962. 14. 537—544.
- HANNA, C., A demonstration of morphine tolerance and physical dependence. *Arch. int. Pharmacodyn.* 1960. 124. 326—329.
- HAYAMA, T., II. Untersuchungen über die Adrenalinsekretion bei Kaninchen. *Jap. J. med. Sci.* 1932. 4. 41.
- HERKEN, H., D. NEUBERT and R. TIMMLER, Die enzymatische N-Demethylierung durch Leber-Mikrosomen bei der Morphin-Gewöhnung. *Naunyn-Schmiedeberg's Arch. exp. Path. u. Pharmak.* 1959. 237. 319—333.
- HESS, W. R., *Das Zwischenhirn*. Basel. 1954.
- HIMMELSBACH, C. K., G. H. GERLACH and E. J. STANTON, A method for testing addiction, tolerance and abstinence in the rat. *J. Pharmacol. exp. Ther.* 1935. 53. 179—188.
- HIMMELSBACH, C. K., IV. With reference to physical dependence. *Fed. Proc.* 1943. 2. 201—203.
- HÖKFELT, B., Noradrenaline and adrenaline in mammalian tissues. *Acta physiol. scand.* 1951. 25. Suppl. 92.
- HOLTZ, P., K. CREDNER und G. KRONEBERG, Über das sympathicomimetische pressorische Prinzip des Harns ("Urosympathin"). *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* 1947. 204. 228—243.
- HOLTZ, P., Über die sympathicomimetische Wirksamkeit von Gehirnextrakten. *Acta physiol. scand.* 1950. 20. 354—362.
- HOLTZ, P. and E. WESTERMANN, Über die Dopadecarboxylase und Histidindecaboxylase des Nervengewebes. *Naunyn-Schmiedeberg's Arch. exp. Path. u. Pharmak.* 1956. 227. 538—546.
- HOLTZ, P., H. BALZER, E. WESTERMANN und E. WEZLER, Beeinflussung der Evipannarkose durch Reserpin, Iproniazid und biogene Amine. *Naunyn-Schmiedeberg's Arch. exp. Path. u. Pharmak.* 1957. 231. 333—348.
- HOLZBAUER, M. and M. VOGT, Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.* 1956. 1. 8—11.
- ISBELL, H. and H. F. FRASER, Addiction to analgesics and barbiturates. *Pharmacol. Rev.* 1950. 2. 355—397.
- JOEL, E. und A. ETTINGER, Zur Pathologie der Gewöhnung. III. Mitteilung: Experimentelle Studien über Morphingewöhnung. *Naunyn-Schmiedeberg's Arch. exp. Path. u. Pharmak.* 1926. 115. 334—350.
- JOHANNESSON, T., Morphine as an inhibitor of brain cholinesterases in morphine-tolerant and non-tolerant rats. *Acta pharmacol. (Kbh.)* 1962. 19. 23—35.
- KAAHA, B. R., Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of rhinencephalic and other structures in primates cat and dog. *Acta physiol. scand.* 1951. 24. Suppl. 83.
- KÄRKI, N., R. KUNTZMAN and B. B. BRODIE, Storage, synthesis and metabolism of monoamines in the developing brain. *J. Neurochem.* 1962. 2. 53—58.
- KAYMAKALAN, S. and L. A. WOODS, Nalorphine-induced "abstinence syndrome" in morphine-tolerant albino rats. *J. Pharmacol. exp. Ther.* 1956. 117. 112—116.
- KIRSHNER, N. and McC. GOODALL, Separation of adrenaline, noradrenaline and hydroxytryptamine by ion exchange chromatography. *J. Biol. Chem.* 1957. 226. 207—212.
- KOLB, L. and K. HIMMELSBACH, Methods of study of the abstinence syndrome. *Amer. J. Psychiat.* 1938. 94. 759—799.
- KRUEGER, H., N. B. EDDY and M. SUMWALT, The pharmacology of the opium alkaloids. *Publ. Health. Rep.* 1941. Suppl. 165.

- LEDUC, J., Catecholamine production and release in exposure and acclimation to cold. *Acta physiol. scand.* 1961. 53. Suppl. 183.
- LEROY, J. G. and A. F. de SCHAEFDYVER, Urine and catecholamine output in normal and reserpinized dogs. *Arch. int. Pharmacodyn.* 1961. 130. 437—454.
- LEWIS, C. E., The biological actions of vanadium. *Arch. Industr. Health.* 1959. 20. 455—466.
- LUND, A., Fluorimetric determination of adrenaline in blood. III. A new sensitive and specific method. *Acta pharmacol. (Kbh)* 1949. 5. 231—247.
- LUND, A., Simultaneous fluorimetric determinations of adrenaline and noradrenaline in blood. *Acta pharmacol. (Kbh.)* 1950. 6. 137—146.
- MACFARLANE, P. S., C. E. DALGLIESH, R. W. DUTTON, L. LENNOX, L. M. NYHUS and A. N. SMITH, Endocrine aspects of argentaffinoma with special reference to the use of urinary 5-hydroxyindoleacetic acid estimations in diagnosis. *Scot. med. J.* 1956. 1. 148—155.
- MAGGIOLO, C. and F. HUIDBORO, The influence of some drugs on the abstinence syndrome to morphine in mice. *Arch. int. Pharmacodyn.* 1962. 138. 157—168.
- MAYNERT, E. W., G. I. KLINGMAN, Tolerance to morphine. I. Effects on catecholamines in the brain and adrenal glands. *J. Pharmacol. exp. Ther.* 1962. 135. 285—295.
- MAYNERT, E. W., G. I. KLINGMAN and H. K. KAJI, Tolerance to morphine. II. Lack of effects on brain 5-hydroxytryptamine and γ -aminobutyric acid. *J. Pharmacol. exp. Ther.* 1962. 135. 296—299.
- MILTHERS, K., N-Dealkylation of morphine and nalorphine in the brain of living rats. *Nature (Lond.)* 1962. 195. 607.
- MONTAGU, K. A., Adrenaline and noradrenaline concentration in rat tissues. *Biochem. J.* 1956. 63. 559—565.
- MONTAGU, K. A., Seasonal variations of noradrenaline and adrenaline concentrations in rat tissues. *Nature (Lond.)* 1956. 178. 417—419.
- MONTAGU, K. A., Catechol compounds in rat tissues and in brains of different animals. *Nature (Lond.)* 1957. 180. 244—245.
- MULÉ, S. J. and L. A. WOODS, Distribution of N-C¹⁴-methyl labeled morphine: I. In central nervous system of non-tolerant and tolerant dogs. *J. Pharmacol. exp. Ther.* 1962. 136. 232—241.
- MYERS, H. B., The effect of chronic morphine poisoning upon growth, the oestrus cycle and fertility of the white rat. *J. Pharmacol. exp. Ther.* 1931. 41. 317—323.
- NETTER, K. J., V. H. COHN and P. A. SHORE, Sex difference in histamine metabolism in the rat. *Amer. J. Physiol.* 1961. 201. 224—226.
- OGURI, A., Quantitative changes of spontaneous activity of rats due to repeated administration and abrupt withdrawal of morphine. *Fol. pharmacol. japon.* 1961. 57. 25—26.
- OUTSCHOORN, A. S., The hormones of the adrenal medulla and their release. *Brit. J. Pharmacol.* 1952. 7. 605—615.
- PAASONEN, M. K. and M. VOGT, The effect of drugs on the amounts of substance P and 5-hydroxytryptamine in mammalian brain. *J. Physiol. (Lond.)* 1956. 131. 617—626.
- PAROLI, E. and P. MELCHIORRI, Urinary excretion of hydroxysteroids, 17-ketosteroids and aldosterone in rats during a cycle of treatment with morphine. *Biochem. pharmacol.* 1961. 6. 1—17.
- PERMAN, E. S., Effect of ethanol and hydration on the urinary excretion of adrenaline and noradrenaline and on the blood sugar of rats. *Acta physiol. scand.* 1961. 51. 68—74.
- PLANT, O. H. and I. H. PIERCE, Studies of chronic morphine poisoning in dogs: I. General symptoms and behavior during addiction and withdrawal. *J. Pharmacol. exp. Ther.* 1928. 33. 329—357.

- PLETSCHER, A., P. A. SHORE and B. B. BRODIE, Release of brain serotonin by reserpine. *J. Pharmacol. exp. Ther.* 1956 a. 116. 46.
- PLETSCHER, A., P. A. SHORE and B. B. BRODIE, Serotonin as a mediator of reserpine action in brain. *J. Pharmacol. exp. Ther.* 1956 b. 116. 84—89.
- PLETSCHER, A. and K. F. GEY, Drug-induced alterations of the metabolism of cerebral monoamines. *Monoamines et système nerveux central*. Genève 1962. p. 105—115.
- QUINN, G. P., B. B. BRODIE and P. A. SHORE, Drug-induced release of norepinephrine in cat brain. *J. Pharmacol. exp. Ther.* 1958. 122. 63 A.
- RANGAPPA, K. S., Cryoscopic effect of separation and concentration of solutes. *Nature (Lond.)*. 1961. 191. 1087—1088.
- RENSON, J., Excrétion urinaire d'acide 5-hydroxyindolacétique chez le Mammifère irradié. *J. Physiol. (Paris)*. 1960. 52. 208—209.
- REYNOLDS, A. K. and L. O. RANDALL, *Morphine and allied drugs*. University of Toronto Press. Toronto 1957.
- RICHARDSON, J. A., E. F. WOODS, A. K. RICHARDSON, Elevations in plasma catecholamines following morphine. *J. Pharmacol. exp. Ther.* 1958. 122. 64 A.
- ROOS, B.-E. and B. WERDINIUS, Effect of reserpine on the level of 5-hydroxyindoleacetic acid in brain. *Life Sciences*. 1962. 3. 105—107.
- ROSENGREN, E., Are dihydroxyphenylalanine decarboxylase and 5-hydroxytryptophan decarboxylase individual enzymes? *Acta physiol. scand.* 1960 a. 49. 364—369.
- ROSENGREN, E., On the role of monoamine oxidase for the inactivation of dopamine in brain. *Acta physiol. scand.* 1960 b. 49. 370—375.
- SATO, H. and F. OHMI, Action of morphine on the epinephrine output, blood sugar content and blood pressure in dogs. *Tohoku J. exp. Med.* 1933. 21. 411—432.
- SCHAPIRO, S., Effect of a catecholamine blocking agent (dibenzylamine) on organ content and urine excretion of noradrenaline and adrenaline. *Acta physiol. scand.* 1958. 42. 371—375.
- SCHAUMANN, O., Morphin und morphinähnlich wirkende Verbindungen. *Handbuch d. Exp. Pharmacol.* 1957. 12.
- SEEVERS, M. H., Opiate addiction in the monkey. I. Methods of study. *J. Pharmacol. exp. Ther.* 1936. 56. 147—156.
- SEEVERS, M. H., Adaptation to narcotics. *Fed. Proc.* 1954. 13. 672—684.
- SEGAL, M. and G. A. DENEAU, Brain levels of epinephrine (E), norepinephrine (N), dopamine (D) and 5-HT during administration and withdrawal of morphine in monkeys. *Fed. Proc.* 1962. 21. 327.
- SHORE, P. A. and B. B. BRODIE, LSD-Like effects elicited by reserpine in rabbits pretreated with iproniazid. *Proc. Soc. exp. Biol. (N. Y.)*. 1957. 94. 433—435.
- SHORE, P. A., J. A. R. MEAD, R. C. KUNTZMAN, S. SPECTOR and B. B. BRODIE, On the physiologic significance of monoamine oxidase in brain. *Science* 1957. 126. 1063—1064.
- SIBUTA, H., K. ENDO and G. NAGAKURA, The increase of epinephrine output under morphine depends upon the integrity of the splanchnic nerves. *Tohoku J. exp. Med.* 1949. 50. 1—6.
- SLOAN, J., A. J. EISENMAN, J. W. BROOKS and W. R. MARTIN, Catecholamine and serotonin levels in tissues of morphine addicted rats following abrupt withdrawal. *Fed. Proc.* 1962 a. 21. 326.
- SLOAN, J., J. W. BROOKS, A. J. EISENMAN and W. R. MARTIN, Comparison of the effects of single doses of morphine and thebaine on body temperature, activity and brain and heart levels of catecholamines and serotonin. *Psychopharmacologia (Berl.)*. 1962 b. 3. 291—301.
- SOLLMAN, T., Studies of chronic intoxications on albino rats. *J. Pharmacol. exp. Ther.* 1924. 23. 449—459.
- SOURKES, T. L., G. F. MURPHY, B. CHAVEZ and M. ZIELINSKA, The action of some

- α -methyl and other amino acids on cerebral catecholamines. *J. Neurochem.* 1961. 8. 109—115.
- SPECTOR, S., R. KUNTZMAN, P. A. SHORE and B. B. BRODIE, Evidence for release of brain amines by reserpine in presence of monoamine oxidase inhibitors. Implication of monoamine oxidase in norepinephrine metabolism in brain. *J. Pharmacol. exp. Ther.* 1960. 130. 256—261.
- SPECTOR, S., K. MELMON and A. SJOERDSMA, Evidence for a rapid turnover of norepinephrine in rat heart and brain. *Proc. Soc. exp. Biol. (N. Y.)*. 1962. 111. 79—81.
- STEWART, G. N. and J. M. ROGOFF, The action of drugs on the output of epinephrine from the adrenals. *J. Pharmacol. exp. Ther.* 1922. 19. 59—85.
- STOLZ, F., Über Adrenalin und Alkylaminoacetobrenzcatechin. *Ber. Deutsch. Chem. Ges.* 1904. 37. 4149—4154.
- SULSER, F. and B. B. BRODIE, Is reserpine tranquilization linked to change in brain serotonin or brain norepinephrine? *Science*. 1960. 131. 1440—1441.
- TACHIKAWA, Y., Effect of morphine injections on blood-sugar and adrenaline in dogs. *J. Orient. Med.* 1932. 17. 521—528.
- TADA, H., Effect of morphine upon the blood sugar content of rabbits deprived of either splanchnic nerves or suprarenals. *Tohoku J. Exp. Med.* 1932. 19. 405—420.
- TAKAMINE, J., The isolation of the active principle of the suprarenal gland. *J. Physiol. (Lond.)*. 1901. 27. 29P—30P.
- TAKEMORI, A. E. and G. J. MANNERING, Metabolic N- and O-demethylation of morphine and morphinan-type analgesics. *J. Pharmacol. exp. Ther.* 1958. 123. 171—179.
- TANABE, T. and E. J. CAFRUNY, Adrenal hypertrophy in rats treated chronically with morphine. *J. Pharmacol. exp. Ther.* 1958. 122. 148—153.
- TITUS, E. and S. UDENFRIEND, Metabolism of 5-hydroxytryptamine (serotonin). *Fed. Proc.* 1954. 13. 411.
- TONINI, G., G. MISSERE and M. BABBINI, Ricerche actografiche ed elettroforetiche sul morfinismo nel ratto. *Arch. ital. Sci. farmacol.* 1959. 9. 425—456.
- TWAROG, B. M. and I. H. PAGE, Serotonin content of some mammalian tissues and urine and a method for its determination. *Amer. J. Physiol.* 1953. 175. 157—161.
- UDENFRIEND, S., H. WEISSBACH and C. T. CLARK, The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. *J. Biol. Chem.* 1955. 215. 337—344.
- UDENFRIEND, S., E. TITUS and H. WEISSBACH, The identification of 5-hydroxy-3-indoleacetic acid in normal urine and a method for its assay. *J. Biol. Chem.* 1955. 216. 499—505.
- UDENFRIEND, S., D. BOGDANSKI and H. WEISSBACH, Increase in tissue serotonin by administration of its precursor, 5-hydroxytryptophan. *Fed. Proc.* 1956. 15. 493.
- UDENFRIEND, S., H. WEISSBACH and D. F. BOGDANSKI, Biochemical findings relating to the action of serotonin. *Ann. N. Y. Acad. Sci.* 1957. 66. 602—608.
- UDENFRIEND, S., Amine metabolism and its pharmacological implications. *Neuropharmacology*. Transact. V's conference. Ed. Abramson. New York 1959.
- UDENFRIEND, S., R. CONNAMACHER and S. M. HESS, On the mechanism of release of norepinephrine by α -methyl-m-tyrosine and α -methyl-m-tyramine. *Biochem. Pharmacol.* 1961. 8. 419—424.
- UDENFRIEND, S., *Fluorescence assay in biology and medicine*. Academic Press. New York and London 1962.
- WADA, M., H. TANAKA, T. HIRANO and Y. TANEITI, The effect of morphine administration upon the output rate of epinephrine, blood sugar level and blood pressure in normal and tolerant dogs. *Tohoku J. exp. Med.* 1938. 34. 52—71.
- WEEKS, J. A., Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. *Science* 1962. 138. 143—144.
- WELSH, J. H., Hydroxytryptamine: a neurohormone in the invertebrates. *Fed. Proc.* 1954. 13. 162—163.

- WEIL-MALHERBE, H. and A. D. BONE, Effect of reserpine on the intracellular distribution of catecholamines in the brain stem of the rabbit. *Nature (Lond.)*, 1958, *181*, 1474—1476.
- WEIL-MALHERBE, H., J. AXELROD and R. TOMCHICK, Blood-brain barrier for adrenaline. *Science* 1959, *129*, 1226—1227.
- WEST, G. B., The stability of noradrenaline solutions. *J. Pharm. and Pharmacol.* 1952, *4*, 560—565.
- WILSON, C. W. M. and B. B. BRODIE, The absence of blood-brain barrier from certain areas of the central nervous system. *J. Pharmacol. exp. Ther.* 1961, *133*, 332—334.
- VOGT, M., The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol. (Lond.)* 1954, *123*, 451—481.
- VOGT, M., Sympathomimetic amines in the central nervous system. *Brit. med. Bull.* 1957, *13*, 166—171.
- VOGT, M., Catecholamines in brain. *Pharmacol. Rev.* 1959, *11*, 483—489.
- WOOLLEY, D. W. and E. SHAW, A biochemical and pharmacological suggestion about certain mental disorders. *Proc. Natl. Acad. Sci. (Wash.)* 1954, *40*, 228—231.
- ZELLER, E. A. and J. BARSKY, In vivo inhibition of liver and brain monoamine oxidase by 1-isonicotinyl-2-isopropyl hydrazine. *Proc. Soc. exp. Biol. (N. Y.)* 1952, *81*, 459—461.

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STOCKHOLM 1963